JOURNAL OF AGRICULTURAL RESEARCH

VOL. XXVIII WASHINGTON, D. C., April 19, 1924 No. 3

THE RELATION OF ANTECEDENT EGG PRODUCTION TO THE SEX RATIO OF THE DOMESTIC FOWL[1]

By M. A. JULL

Senior Poultryman, Bureau of Animal Industry, United States Department of Agriculture

INTRODUCTION

In the domestic fowl comparatively little work has been done to determine the normal sex ratio and still less has been done to determine what factors might operate toward a possible modification of the normal sex ratio. As a contribution in this direction an experimental study was undertaken to determine the sex ratio in the domestic fowl from the time of commencing to lay to practically the end of the first year of production. Under the direction of Dr. L. J. Cole, of the department of genetics of the University of Wisconsin, the plan of the work was originally outlined in 1919 and was continued for three consecutive years. Among other things the plan of work involved a study of the following topics:

1. The normal sex ratio.
2. The relation of prenatal mortality to the sex ratio.
3. The relation of egg production to the sex ratio.
4. The relation of egg weight to the sex ratio.
5. The relation of yolk weight to the sex ratio.
6. The relation of egg water content to the sex ratio.

In this paper it should be understood that the term "sex determination" has reference to the causes which lead to the production of an individual of one or the other sex. During recent years considerable progress has been made in arriving at a more complete understanding of the causes which determine sex but this does not necessarily imply an increased ability to control sex. The term "sex control" is understood to imply that the causes which determine sex are more or less amenable to human control.

There are many investigators who believe that the presence or absence of a second sex chromosome constitutes one link only in a series of events that precede the development of sex. The opinions of these investigators range from those in which the sex chromosomes are regarded as playing an insignificant rôle in sex determination to those in which the internal mechanism is regarded as being really responsible for the control of sex but where external conditions may be operative to a minor extent in modifying sex development. The question naturally arises as to whether sex determination involves an irreversible tendency to the corresponding sex differentiation, or whether such differentiation may be controlled or even reversed.

[1] Received for publication April 26, 1924. The experimental part of the work was conducted in the department of genetics, Agricultural Experiment Station, University of Wisconsin, and at Macdonald College, St. Anne d'Bellevue, P. Q., Canada. The study was completed in the U. S. Department of Agriculture. Published with the approval of the director of the Wisconsin station as Paper No. 46.

EXPERIMENTAL STUDY OF THE SEX RATIO IN THE DOMESTIC FOWL

MATERIALS

During the first year of this experimental study reciprocal matings were made between Barred Plymouth Rocks and single-comb White Leghorns. Two pens of birds were used, each pen consisting of five Barred Plymouth Rock and five White Leghorn pullets, the first pen being mated to a Barred Plymouth Rock cockerel and the second to a White Leghorn cockerel. In the matings between Barred Plymouth Rocks and White Leghorns all the chicks were either entirely or largely white, the white of the White Leghorn being dominant to the sex-linked barring factor of the Barred Plymouth Rock.

During the last two years matings were made between Barred Plymouth Rock pullets and single-comb Brown Leghorn cockerels. The mating for the year 1920–21 consisted of 10 pullets and 1 cockerel and for the year 1921-22 two pens were mated, each consisting of 10 pullets and 1 cockerel. In these matings the sex-linked barring factor of the Barred Plymouth Rock is dominant to the Brown Leghorn plumage coloration. The male progeny is always barred, with shanks of the normal yellow color of both parental breeds. The females are always black or black tinged with brown, with black-pigmented shanks. At hatching time it is very easy to distinguish the sexes, since the males invariably have a small patch of white down on the back of the head and the shanks are always light in color, while the females have no white patch on the back of the head and the shanks are always black or very dark in color. The identification of the sex at hatching time is quite reliable, and during the two years out of 1,370 chicks only six errors were made in identifying the sex prior to dissection or observation of the secondary sexual characters of the growing birds.

As far as the observations in this study are concerned, it can be said that external appearances of the chicks at hatching time give no indication of reversibility of sex. The characteristic secondary sexual features of the male were always associated with a male as shown by dissection, and so with all females. Birds which were allowed to reach the adult stage showed no signs of an intermediate sex condition or of changing from one sex to another.

Of the 50 females used in this study, the records of only 45 are analyzed, since either through early death or very poor production the records of 2 birds for each of the first two years have been discarded and in the third year female No. 1508 produced not 1 fertile egg out of 63 laid.[3]

METHODS

All of the birds were trap-nested throughout each year. Complete records were kept of production, and during the last two years the eggs were weighed daily. Throughout the three years the eggs were placed in the incubators on an average of about every 10 days. From the time of gathering the eggs to the conclusion of the work each season practically every egg has been accounted for. All cracked eggs have been recorded, whether they were cracked by the hen in the nest, by the attendant in gathering the eggs, or by the operator during the period of incubation. Detailed records were kept of all eggs which were infertile, embryos which died before the twelfth day of incubation and on which sex could not readily be obtained, embryos which died on and after the twelfth day

[3] The first and last year's work was done in the department of poultry husbandry of Macdonald College, while the second year's work was done in the department of genetics of the University of Wisconsin, the birds being very kindly supplied by Prof. J. G. Halpin, of the department of poultry husbandry of that institution.

of incubation and on most of which the sex was determined. During the normal hatching season the chicks which hatched were banded, and a note was made of the sex, which was later checked by observation of the secondary sexual characters. Up to and including the time of the normal hatching season, the chicks were allowed to hatch, and whatever chicks hatched were killed and dissected immediately. Subsequent to the hatching season the eggs was removed from the incubator about the nineteenth day and were all dissected. Occasionally an egg would become lost, usually through being broken in attending the incubator and no record being made. The sex on a few chicks was undetermined, either through two or more chicks getting together in the hatching trays or through chicks becoming lost during the growing season. Although, under normal conditions, the sources of loss of records among growing chicks are numerous, including loss from enemies of the poultry flock and loss of legband before the insertion of the permanent wing band, the number of eggs and chicks in this experiment on which no records were obtained was small indeed Furthermore, it is probably true that all cracked and missing eggs and all missing chicks would constitute a random sample of the entire population as far as the matter of sex ratio is concerned.

The weights of the eggs are considered in relation to production and to sex ratio. Using another group of birds, the weights of the yolks of all eggs laid during the first year of production have been determined and are considered in relation to production and to sex ratio. The water content of a number of eggs laid at different periods of production has been determined, and this also is considered in relation to production and to sex ratio.

RESULTS

Before undertaking the presentation of the results from this experimental study, it is desirable to call attention to one or two matters. The sex ratio is presented in terms of the percentage of the males in the total population. In subsequent sections of this paper the sex ratio will be given in terms of the male ratio and will de designated as R♂.

In the context which follows the following symbols have the attached significance: R♂H=per cent of males in all chicks which hatched; R♂D=per cent of males among all dead embryos in which sex was determined.

TABLE I.—*Summary table showing complete results of the three-year period 1919–22*

Year	Number of birds	Total egg production	Cracked eggs	Infertile eggs	Dead embryos not sexed	Missing eggs and chicks	Total females	Total males	Total sexed	Sex ratio
1919–20	18	1,845	116	132	564	7	530	476	1,026	46.39±0.64
1920–21	8	979	57	217	199	5	246	255	501	50.89±0.85
1921–22	19	1,779	82	551	251	26	440	429	869	49.37±0.66
Total	45	4,603	255	900	1,014	38	1,236	1,160	2,396	48.41±0.47
Per cent			5.54	19.55	22.03	0.82	26.85	25.20	52.05	

Table I gives the mean sex ratio of the experimental fowls for the whole three years. It will be noticed that the sex ratio for the second year is higher than for either of the other two years, but it should be explained that during the second year two of the eight birds gave very high male ratios throughout the year.

The question which arises is whether the sex ratio of 48.41±0.47 represents the normal sex ratio for the domestic fowl, since this ratio is determined upon the total production for the first year, whereas most of the sex ratios presented in biological literature deal with ratios determined during the normal hatching season only.

1. THE NORMAL SEX RATIO

The term "normal sex ratio" is understood to mean the sex ratio obtained during the normal hatching season, which may be considered to include approximately the months of March and April.

Pearl (*44*)[3] has indicated the advantages, from a methodological viewpoint, of treating sex-ratio data from the standpoint of the family as the unit and, furthermore, he has shown quite clearly that the value of the male ratio, based on families of 10 or more "is much nearer the true biological norm" than values based upon the population as a whole and on families of less than 10. For instance, he obtained the following ratios: Families of all sizes, R♂=49.45; families of from one to three R♂=55.07±2.11; families of from four to nine, R♂=49.39±0.84; families of 10 or more, R♂=48.57±0.28. Pearl has shown that size of family has an obvious influence on the sex ratio, and he also points out that "families under 10 can not be considered as representative of the normal fertility of the *domestic* fowl."

Taking Pearl's basis as the correct one on which to determine the mean sex ratio of the domestic fowl for portions of the annual production, and assuming that the value 48.57±0.28 was obtained for the normal hatching season, which in the State of Maine would include approximately the months of March and April, the data in this study have been arranged to show the mean sex ratio for the months of March and April and for the preceding and subsequent seasons of production, all ratios being computed on the basis of families of 10 or more.

TABLE II.—*Showing the mean sex ratio for the normal hatching season and for the preceding and subsequent seasons of production. Based on families of 10 or more*

Period No.	Year	Females	Males	Sex ratio	Differences
I (from the commencement of laying to the end of February)	1919–20	108	160	59.70±1.97	
	1920–21	68	89	56.69±1.53	
	1921–22	177	198	52.80±1.18	
Total		353	447	55.87±0.98	I–II 7.05±1.22
II (normal hatching season, March and April)	1919–20	178	160	47.34±0.83	
	1920–21	87	102	53.97±2.16	
	1921–22	214	195	47.68±0.93	
Total		479	457	48.82±0.80	II–III 12.00±1.01
III (From the first of May to the end of October)	1919–20	247	142	36.50±0.86	
	1920–21	91	55	37.67±1.42	
Total		338	197	36.82±0.61	I–III 19.05±1.15

It should be mentioned here that the sex ratios based on the population as a whole for each of the three periods shown in Table II have been found to be as follows: Period No. I, from the commencement of laying to the end of February, R♂=55.95±1.70; Period No. II, normal hatching season, March and April, R♂=49.01±1.03; Period No. III, from the first of May to the end of October, R♂=36.93±0.87. When compared with the sex ratios determined for the same periods based on families of 10 or more, 55.87±0.98, 48.82±0.80, and 36.82±0.61, as shown in Table II, it is apparent that the values of the ratios based on families of 10 or more show less variability.

From Table II it is evident that the sex ratio for the normal hatching season, R♂=48.82±0.80, is comparable to the value, R♂=48.57±0.28, as determined by Pearl. It should be remembered that Pearl was dealing with much larger numbers. The difference is not significant, however, since the difference with its probable error is 0.25±0.85. At the same time, it may be pointed out that this

[3] Reference is made by number (italic) to "Literature cited," pp. 221–224.

is the first study to be conducted on a determination of the sex ratio in the domestic fowl based on an entire year's production, and it was practically impossible to use a large number of birds.

The most interesting fact which Table II brings out is the high sex ratio, R♂=55.87±0.98, in the period of production preceding the normal hatching season and the low sex ratio, R♂=36.82±0.61, in the period of production subsequent to the normal hatching season. The sex ratios for each year show a fairly regular decrease from period to period. The only apparent exception in the magnitude of decrease occurs in connection with the sex ratio in period No. II, year 1920–21, as compared with period No. I, for the same year, and this can be explained by the presence of two birds giving very high male ratios, No. 191, R♂=91.67 and No. 197, R♂=66.67.

The difference between the sex ratios of any two periods is significant and appears to be due to specific genetic or physiological factors, the probable character of which is discussed later.

2. THE RELATION OF PRENATAL MORTALITY TO THE SEX RATIO

Up to the present, the problem of the sex ratio in the domestic fowl has been considered only in terms of the relation of total numbers of sexes determinable, no distinction being made between the sexes of chicks that hatched and the sexes of the dead embryos. Notwithstanding the fact that practically every egg in all the families has been accounted for, the sex ratio at hatching time does not necessarily indicate the relative proportions of the initially produced zygotes, because there may have been a differential mortality of the sexes in the embryos which died up to the twelfth day of incubation and in the embryos which died between the twelfth and twenty-first days of incubation. In other words, the prenatal mortality may have effected a deviation from the initial zygotic ratio.

It has been demonstrated that the mean sex ratio of the population as a whole, is 48.41±0.47 and that the mean sex ratio, based on families of 10 or more for the period from the commencement of laying to the end of February, is 55.87±0.98, for the period of the normal hatching season, March and April, is 48.82±0.80, and for the period from the first of May to the end of October is 36.82±0.61. Two of these sex ratios show a marked deviation from equality and the problem arising out of such a situation is to determine whether a possible differential mortality has served as a cause in affecting the deviations.

The first step in an analysis of this problem is to determine, if possible, the proportions in which zygotes of the two sexes are initially produced. It is obvious that the cracked eggs and the missing eggs and chicks and probably the infertile eggs would constitute a random sample of the population as far as the sex ratio is concerned. There remain for consideration, therefore, the fertile eggs. The sex of the zygote can not be distinguished until a certain stage of embryonic development has been reached, and in this study, while the sex was determined on some younger embryos, the author felt less possibility of error when using the twelfth day as the basis of earliest determination. On the other hand, the sex could not be determined on some dead embryos in relatively advanced stages of development because of degeneration of the gonads between the time of death and the time of dissection. But the number falling under this category was very small and could not materially affect either the zygotic ratio at the time of fertilization or the sex ratio at hatching time.

Regarding the embryos which died before the twelfth day of incubation, it is impossible to determine whether or not the mortality is differential. Regarding the embryos which died after the eleventh day, accurate records have been kept, which are presented in Table III, including from the commencement of laying

to the close of the normal hatching season. The matter of bagging and recording the eggs individually on the nineteenth day of incubation in preparation for hatching requires considerably more time and trouble than simply removing the eggs from the incubators on the nineteenth day for dissecting all chicks in the shell. This is the only reason that no actual hatching was allowed to take place after the hatching season was over. It is realized now, however, that the death rate of each sex for the season subsequent to the normal hatching season might have some significance, and it is anticipated that data on this point will be made available in the near future.

Table III shows for the two periods (No. I), from the commencement of laying to the end of February, and (No. II) the normal hatching season, March and April, (1) the number of embryos dead before the twelfth day and in which sex was not determined, (2) the per cent these constitute of the total number of embryos, both dead and hatched, i. e., of fertile eggs, (3) the number of female embryos dead between the eleventh day and hatching, (4) the number of male embryos dead, (5) the number of females hatched, (6) the number of males hatched, (7) per cent males in embryos dead after eleventh day, (8) per cent males in chickens hatched, (9) per cent males of all sexes determined.

TABLE III.—*Showing the relation of prenatal mortality to the sex ratio, based on families of 10 or more*

PERIOD NO. I.—FROM THE COMMENCEMENT OF LAYING TO THE END OF FEBRUARY

Year	Embryos dying before twelfth day		Embryos dying after eleventh day		Hatched		Sex ratio of embryos dying after eleventh day	Sex ratio of chicks hatched	Sex ratio
	Number	Per cent of all embryos	Females	Males	Females	Males			
1919–20	126	31.97	47	34	61	126	41.97±3.98	67.38±1.11	59.70±2.02
1920–21	75	32.33	27	27	41	62	50.00±3.74	60.19±1.24	56.69±1.53
1921–22	98	20.78	72	48	105	150	47.00±1.43	58.82±1.06	52.80±1.18
Total	299	27.20	146	109	207	338	47.74±2.02	62.02±0.74	55.87±0.98

PERIOD NO. II.—NORMAL HATCHING SEASON, MARCH AND APRIL

1919–20	143	29.73	65	45	113	115	45.91±2.88	50.44±1.04	47.34±0.83
1920–21	32	14.48	31	30	56	72	49.18±2.14	56.25±1.67	53.97±2.16
1921–22	87	17.74	73	45	141	150	48.13±2.53	51.55±1.51	47.68±0.93
Total	262	21.87	169	120	310	337	46.52±1.71	52.09±0.89	48.82±0.80

Taking Table III as a whole, four things stand out prominently: (1) The percentage of embryos dying before the twelfth day fluctuates from year to year in both periods, but for each year the percentages are lower in period No. II than in period No. I; (2) with the exception of the year 1920–21, there were slightly fewer males than females that died after the eleventh day in period No. I as compared with period No. II, the percentages for the two periods being respectively 47.74 ± 2.02 and 46.52 ± 1.71; (3) in every case there were more males than females that hatched, but for period No. I the proportion was much higher than for period No. II, i. e., 62.02 ± 0.74 as compared with 52.09 ± 0.89; (4) in each year in period No. I the percentage of males of all sexes determined was relatively high, that for the period as a whole being 55.87 ± 0.98, while in each year except 1920–21 in period No. II the percentage of males was below fifty, that for the period as a whole being 48.82 ± 0.80.

The percentage of embryos dying before the twelfth day is of sufficient magnitude in any population so that if they were all of the same sex and if they were added to the deficient sex based on the observed ratios each observed ratio would be reversed.

In Table IV the data for periods Nos. I, II, and III are arranged to show the sex ratios required in the embryos dying before the twelfth day to produce an equality of sexes in the total population, based on families of 10 or more. On this assumption, the results in the last column of Table IV show that for the three periods there would have to be a successive increase in the percentage of males in the embryos dying before the twelfth day. Period No. III shows an extremely high assumed sex ratio of embryos dying before the twelfth day, far higher than could reasonably be expected, particularly in view of other evidence presented. Moreover, the assumed ratios of embryos dying before the twelfth day for periods Nos. I and II are each considerably different in value from the observed ratios of embryos dying after the eleventh day.

TABLE IV.—*Showing the sex ratios required in the embryos dying before the twelfth day to produce an equality of sexes in total population, based on families of 10 or more*

Period No.	Embryos dying after eleventh day		Chicks hatched		Observed sex ratios of embryos dying after eleventh day	Observed sex ratios of chicks hatched	Distribution of embryos dying before twelfth day to produce an equality of sexes in total population		Assumed sex ratio of embryos dying before twelfth day to produce an equality of sexes in total population
	Females	Males	Females	Males			Females	Males	
I	146	109	207	338	47.74	62.02	196.50	102.50	34.28
II	169	120	310	337	46.52	52.09	120.00	142.00	54.20
III	[a] 338	[a] 197	----	----	[a] 36.82	----	103.00	244.00	70.31

[a] Includes all embryos developing up to and after twelfth day, since during this period no chicks were allowed to hatch.

It is obvious, therefore, that the number of embryos dying before the twelfth day is high enough in any population so that if a sufficient majority of the embryos were of one sex the mean sex ratio might be restored to approximate equality. The interesting feature in connection with such a supposition is that in period No. I the differential death rate would have to be considerably in favor of female survivals, since $R\,♂ = 55.87 \pm 0.98$, while in period No. II the differential death rate would have to be in favor of male survivals, since $R\,♂ = 48.82 \pm 0.80$. But the data in Table III show that the percentages of male embryos which died after the eleventh day in both periods are about the same, $R\,♂\ D = 47.74 \pm 2.02$ and $R\,♂\ D = 46.52 \pm 1.71$. In other words, this means that from the time sex could be determined to hatching time there is a relatively higher death rate of females in both periods. Therefore, the higher death rate of females before hatching can not account for the absolute differences in the observed sex ratios between periods Nos. I and II.

In the left half of Table V the embryos dying before the twelfth day are distributed in such a way as to produce sex ratios of equality, and in the fifth column there are given the assumed ratios of these dead embryos. The assumed death rate of these embryos is in striking contrast with the observed death rate of the embryos dying after the eleventh day, as shown in the fifth and sixth columns of

Table V. Since there is no valid reason for supposing a death rate in the sexes of embryos dying before the twelfth day different from a death rate in the sexes of embryos dying after the eleventh day, it may be concluded accordingly that the death rate in the embryos dying before the twelfth day apparently does not affect the mean sex ratio differently from the death rate in embryos dying after the eleventh day. In the right half of Table V there are shown the calculated sex ratios when the death rate of the embryos dying before the twelfth day is assumed to be the same as for the embryos dying after the eleventh day. A comparison of the figures in the last two columns of Table V would indicate a relative difference in the percentage of males produced in period No. I as compared with period No. II.

TABLE V.—*Showing the results obtained when the embryos dying before twelfth day are distributed to produce an equality of sexes and on the basis of the observed sex ratios of embryos dying after the eleventh day; based on families of 10 or more*

PERIOD NO. I.—FROM THE COMMENCEMENT OF LAYING TO THE END OF FEBRUARY

Year	Embryos dying before twelfth day	Distribution of embryos dying before twelfth day to produce an equality of sexes		Assumed sex ratio of embryos dying before twelfth day	Observed sex ratios of embryos dying after eleventh day	Distribution of embryos dying before twelfth day on basis of observed sex ratio of embryos dying after eleventh day		Calculated sex ratio	Observed sex ratio
		Females	Males			Females	Males		
1919-20	126	89.00	37.00	29.36	41.97	73.42	52.58	53.95	59.70
1920-21	75	48.00	27.00	36.00	50.00	37.50	37.50	54.53	56.69
1921-22	98	59.50	38.50	39.28	47.00	51.94	46.06	52.00	52.80
Total	299	196.50	102.50	34.28	47.74	156.26	142.74	53.66	55.87

PERIOD NO. II.—NORMAL HATCHING SEASON, MARCH AND APRIL

1919-20	143	62.50	80.50	56.29	45.91	77.35	65.65	46.91	47.34
1920-21	32	23.50	8.50	26.56	49.18	16.26	15.74	53.28	53.97
1921-22	87	34.00	53.00	60.92	48.13	45.13	41.87	47.76	47.68
Total	262	120.00	142.00	54.20	46.52	150.12	111.88	47.48	48.82

Is there a differential death rate in the embryos dying after the eleventh day? Considering the data in Table III, it will be observed that the percentage of male embryos is lower in every case, except for the year 1920–21, in periods No. I and II, than the mean sex ratio. Pearl (*44, p. 434*) found the sex ratio of the dead embryos for the years 1916 and 1917 to be 48.3 ± 0.77. As between Pearl's ratio and the ratio for period No. I in Table III, the difference with its probable error is 0.56 ± 2.16, and as between Pearl's ratio and the ratio for period No. II in Table III, the difference with its probable error is 1.78 ± 1.87. Nevertheless, the data in Table III seem to show that in period No. I the death rate in embryos dying after the eleventh day does account to a slight extent for the high mean sex ratio, $R\,♂ = 55.87 \pm 0.98$. But the same interpretation can not be placed upon the data in period No. II, since the death rate is practically the same as in period No. I, but the mean sex ratio is considerably lower, $R\,♂ = 48.82 \pm 0.80$. In period No. I, $R\,♂D = 47.74 \pm 2.02$, while in period No. II, $R\,♂D = 46.52 \pm 1.71$. The difference with its probable error is 1.22 ± 2.65 and is, therefore, of no significance. In other words, there is practically no difference in the known prenatal

mortality between period Nos. I and II. Some other explanation must be found for the differential sex ratio existing between the two periods.

Is there a differential hatching rate of the sexes in the two periods? Apparently so, as shown by a comparison of the R♂H and the R♂ columns in Table III. The percentage of males hatching, R♂H, is higher in every case than the percentage of males in the total population, R♂. In period No. I, R♂H = 62.02 ±0.74, while in period No. II, R♂H = 52.09±0.89. The difference with its probable error is 9.93±1.16 and is equally as significant as the difference in sex ratios between the two periods, 7.05±1.22, as indicated previously. It may be said, therefore, that the difference in the mean sex ratios between the two periods is affected primarily by the greater absolute number of males produced in the first period.

These observations lead to the conclusion that the differential sex ratio existing between the period from the commencement of laying to the end of February and the period of the normal hatching season can not be accounted for by a differential prenatal mortality but is accounted for by a differential hatching ratio based upon absolute numbers of the sexes produced. Such a situation rests upon a genetic or physiological interpretation.

3. THE RELATION OF EGG PRODUCTION TO THE SEX RATIO

The nature of the results considered so far suggest a possible correlation between egg production and the sex ratio. In order to ascertain if such is the case the records were gone over carefully and families of 10 or more were selected from each mating, based upon the antecedent egg production. The families were so selected as to eliminate the influence of prenatal mortality as much as possible, in order that each sex ratio determined might represent the highest possible proportion of its total population. The families were grouped into six representative classes, based upon the antecedent egg production. The six classes include those hens which laid from 0 to 20, from 21 to 40, from 41 to 60, from 61 to 80, from 81 to 100, and from 101 to 120 eggs, respectively, prior to selection of the families on which sex ratios were determined. (See Table VI.)

TABLE VI.—*Showing the relation between antecedent egg production and sex ratio*

Class	Antecedent production	Average antecedent production	Embryos dying before twelfth day	Females	Males	Sex ratio	Differences
1	0-20	4.79	25	102	173	62.91±1.44	5.45±2.61
2	21-40	27.86	19	94	127	57.46±1.88	12.46±2.77
3	41-60	47.85	18	165	135	45.00±2.04	0.39±2.15
4	61-80	69.45	12	108	87	44.61±0.69	6.96±1.21
5	81-100	88.92	16	101	61	37.65±1.00	5.45±1.52
6	101-120	114.50	8	80	38	32.20±1.15	

There are four cases in which the sex ratio deviates considerably from the normal of 48.82±0.80, namely, classes 1, 2, 5, and 6. In none of these cases is the number of embryos dying before sex could be determined great enough to account for the deviation in the sex ratio. Furthermore, in the first two cases the numbers of dead embryos would have to be added to the numbers of females and in the last two cases they would have to be added to the numbers of males.

It is apparent that there is a correlation between egg production and sex ratio. Between classes 1 and 2 the difference with its probable error is 5.45 ± 2.61, between classes 2 and 3 it is 12.46 ± 2.77, between classes 3 and 4 it is 0.39 ± 2.15, between classes 4 and 5 it is 6.96 ± 1.21, and between classes 5 and 6 it is 5.45 ± 1.52. The differences are significant in every case except two between classes 1 and 2 and between classes 3 and 4. Between classes 1 and 6 the difference with its probable error is 30.71 ± 1.84, and between classes 2 and 5 the difference with its probable error is 19.81 ± 2.13. The correlation between antecedent egg production and sex ratio is high and is significant, since $r = -0.704 \pm 0.037$. In other words, with the birds used in this study and under the conditions of this study, the conclusion is justified that as egg production increases the sex ratio decreases.

Since it has been demonstrated that a correlation exists between antecedent egg production and sex ratio and since egg production is affected, to some extent at least, by seasonal variations, it becomes necessary to inquire concerning the possibility of a significant correlation between seasonal conditions and sex ratio. In this connection it should be pointed out that of the 45 birds, 3 commenced laying in August, 7 in September, 11 in October, 12 in November, 9 in December, and 3 in January. In view of the widely different times of the year at which the birds commenced laying, it seems logical to assume that if season affects the sex ratio birds commencing to lay early in the season as compared with birds commencing to lay late in the season should give different sex ratios for the first families of 10 or more. In order to test the validity of this assumption the sex ratios of the first families of 10 or more, obtained as nearly as possible from the time laying commenced, and according to the month laying commenced, were tabulated and averages determined. On this basis, the following average sex ratios are as follows: August, 59.17; September, 60.18; October, 61.17; November, 60.91; December, 56.67; January 58.50. It is apparent, therefore, that the season of the year has not affected the sex ratios of the first families of 10 or more.

On the other hand, if season as such affects the sex ratio, then it would be expected that in the antecedent production classes shown in Table VI, each class for which the sex ratio was determined should contain families of 10 or more produced at approximately the same season of the year. In class 1, for instance, antecedent egg production 0–20, in which $R\,♂ = 62.91 \pm 1.44$, it should be expected that the families of 10 or more would have been produced at approximately the same season. But it is found upon examination that the following number of families occurred in the months designated: September, 5; October, 6; November, 7; December, 4; January, 3; February, 1. In class 2, antecedent egg production 21–40, in which case $R\,♂ = 57.46 \pm 1.88$, the following number of families occurred in the respective months: December, 3; January, 6; February, 9; March, 5. These results show quite clearly that season of itself apparently does not directly affect the sex ratio, otherwise irrespective of antecedent production, families occurring at approximately the same season of the year should give sex ratios of approximately the same value.

The possible effect of season on sex ratio can be tested in still another way. On the assumption that season affects sex ratio there should be a significant correlation between the sex ratio of the families and the month in which they occur. The value of this correlation has been determined as follows: $r = -0.165 \pm 0.076$. This correlation is of such low value as not to be significant. Since the correlation between antecedent egg production and sex ratio is -0.704 ± 0.037, and since the correlation between season and sex ratio is -0.165 ± 0.076, it may be concluded that season does not affect the sex ratio unless indirectly through affecting egg production.

Crew and Huxley (*6*) in a study of the sex ratios in the F_1 generation of a Rhode Island Red male Light Sussex female cross observes that as the season advanced the sex ratio decreased. During the spring months, February to June, the sex ratio was 50, while during August and September, the sex ratio was 42.62. The authors state as their opinion that "It is likely that this represents a significant seasonal difference, * * *." In view of the results determined in the present study, however, it seems probable that the modification in sex ratio obtained by Crew and Huxley can be explained on the basis of antecedent egg production.

4. THE RELATION OF EGG WEIGHT TO SEX RATIO

It is quite conceivable that there might be a correlation between egg production and egg weight, and if such were the case then there would also be a correlation between egg weight and sex ratio. If such a correlation were manifest, however, it might or might not be significant.

The author (*28*), in a study of rates of growth in Barred Plymouth Rock chicks, observed a significant difference in the weights of eggs from which the females were obtained as compared with the weights of eggs from which the males were obtained. It should be explained here, however, that the only egg weights taken into consideration were those producing the chicks on which growth rates were determined, the records for analysis being selected at the conclusion of the growth period. The weights of the eggs in which embryos died and the weights of the eggs producing chicks which died prior to the termination of the period of growth studied were not considered. Moreover, it is observed that in the relatively small population of chicks used in the growth study, 38 females and 38 males, there were only 3 families of 10 or more. The difference in the weight of eggs producing the two sexes, 2.06 ± 0.67, can not be considered as evidence bearing on the problem in this study where the weights of eggs producing the two sexes throughout the first year of production have been considered, including the sexes which died between the eleventh and the twenty-first days and the sexes which hatched.

It has been noted previously that for the production years 1920–21 and 1921–22 all eggs were weighed daily as produced, and for each mating the weights have been grouped on the same basis of classification as used in Table VI. The average weights have been determined for the groups 0 to 20, 21 to 40, 41 to 60, 61 to 80, 81 to 100, and 101 to 120 eggs, as produced. Not every group for each mating consisted of 20 eggs. One hen after a long rest might lay 12 eggs in 15 days and then might not lay again for another month. In such a case the average weight of the 12 eggs was determined and allocated to the proper group, according to the antecedent production. Where hens laid more or less continuously for a long period, then the production for that particular period was divided into groups of approximately 20 eggs each. A number of hens laid more than 120 eggs, but the average weights of eggs beyond this production have not been taken into consideration because the numbers of sexes determined were so small as to prevent a true mean sex ratio being determined. The average weights classified as indicated are shown in Table VII.

TABLE VII.—*Showing the relation between egg weight and sex ratio*

Class	Antecedent production	Average egg weight	Sex ratio
		Grams	
1	0– 20	51.80±0.70	62.91±1.44
2	21– 40	53.91±0.69	57.46±1.88
3	41– 60	55.70±0.59	45.00±2.04
4	61– 80	55.15±0.52	44.61±0.69
5	81–100	55.18±0.57	37.65±1.00
6	101–120	53.12±0.52	32.20±1.15

It is apparent from Table VII that the average egg weight increases as egg production increases, until about 60 eggs are laid. Then the average egg weight drops slightly until about 100 eggs are laid, after which there is a more marked drop.

In a study of the nature and causes of changes in egg weight in relation to annual production in 40 Barred Plymouth Rock pullets, the author (*30*) has shown that the earlier in life laying commences the lower is the mean weight of the first 10 eggs laid and the lower is the mean weight of the total production. He has also shown that increase in egg weight is analogous to increase in body weight of the birds laying the eggs, and that the earlier in life laying commences the later in life both maximum body weight and maximum egg weight are attained. In another study, the author[4] has shown that the later in life laying commences the more nearly do the first eggs laid approach the mean egg weight of the total annual production. In other words, among the 59 Barred Plymouth Rock pullets under consideration, it has been shown that those commencing to lay as late as the month of December laid approximately as large eggs at that time as any other time during the first year of production. In view of these observations and in view of the correlation between antecedent production and sex ratio demonstrated to exist in connection with the birds used in this study, it is hardly to be expected that there should exist any significant correlation between egg weight and sex ratio based on annual production.

In Table VII it is shown that there is not more than 4 per cent difference between the average weights of any two contiguous groups, and it is hardly conceivable that such a slight difference should have any significance in this study, particularly since the percentage differences do not follow a regular order, whereas the sex ratios tend to decrease somewhat regularly. Taking into consideration the differences with their probable errors, it is evident that there is no significant relation between egg weight and antecedent egg production and certainly no correlation between egg weight and sex ratio. This point should be comparatively easy to demonstrate, however, if the weights of the eggs giving rise to the two sexes are considered.

TABLE VIII.—*Showing the relation between weights of eggs giving rise to females and males, respectively, and sex ratio*

Class	Production	Average egg weight		Difference	Sex ratio
		Females	Males		
		Grams	*Grams*		
1	0- 20	51.37±0.95	51.45±0.98	0.08±1.36	62.91±1.44
2	21- 40	54.25±0.48	54.32±0.52	0.07±0.71	57.46±1.88
3	41- 60	54.64±0.36	53.93±0.29	0.71±0.46	45.00±2.04
4	61- 80	55.64±0.51	55.40±0.42	0.24±0.66	44.61±0.69
5	81-100	54.56±0.99	54.00±1.12	0.56±1.49	37.65±1.00
6	101-120	52.18±1.21	53.24±1.03	1.06±1.59	32.20±1.15

The difference in weights between the eggs producing the two sexes are not regular, when the production classes are compared. For instance, in the first, second, and sixth classes the average weights of the eggs giving rise to males are slightly in excess of the average weights of eggs giving rise to females, while in the third, fourth and fifth classes the reverse is true. Furthermore, the differences in weights between the sexes are relatively slight and are not at all significant. It may be concluded, therefore, that there is no correlation between egg

[4] JULL, M. A. INCREASE IN EGG WEIGHT IN RELATION TO TIME LAYING COMMENCES. Amer. Nat. (In press.)

weight and sex ratio, and this is hardly to be expected, particularly in view of the fact that over 60 per cent of the absolute weight of the egg constitutes albumen and shell, both secreted around the yolk subsequent to maturation and fertilization.

5. THE RELATION OF YOLK WEIGHT TO SEX RATIO

Since the yolk is a single cell and since the earliest stages of embryonic development take place during the absorption of yolk granules in the growth of the ovum, it is well to inquire if the yolk weight bears any relation to the sex ratio. In order to study this point the weights of the eggs and their yolks laid by seven Barred Plymouth Rock hens of the poultry flock of Macdonald College were determined daily throughout one year of production. These weights are shown in Table IX, along with the sex ratios, for the production classes as shown previously, except that the sixth class is not represented owing to the limited number of eggs produced.

TABLE IX.—*Showing the relation between yolk weight and sex ratio*

Class	Production	Average egg weight	Average yolk weight	Sex ratio
		Grams	*Grams*	
1	0–20	46.33±0.31	15.13±0.23	62.91±1.44
2	21–40	50.07±0.34	15.34±0.26	57.46±1.88
3	41–60	52.40±0.41	15.92±0.28	45.00±2.04
4	61–80	51.08±0.68	15.89±0.27	44.61±0.69
5	81–100	49.16±0.34	15.34±0.26	37.65±1.00

It will be observed from Table IX that apparently there is a correlation bebetween egg weight and yolk weight, the heavier the egg the heavier the yolk. This is a similar result to that observed by Atwood and Weakley, in analyzing the egg records of a number of birds at the West Virginia Experiment Station. The relationship, demonstrated in this study, existing between egg weight and yolk weight, is in close agreement with the results of Atwood and Weakley, but is in disagreement with the results observed by Riddle (*46*, *47*) with pigeons. In a generic cross between *Turtur orientalis* and *Streptopelia alba*, the cross most largely studied, Riddle makes certain observations regarding yolk weight.

> The result clearly establishes the fact that the yolks of late summer and autumn—those that produce mostly, or all, females—are larger than the yolks produced in the spring which give rise to males" (*46*, *p. 389*). "The balances alone and at once showed that the *mass* of the yolk of the first egg of nearly all pairs of eggs (from pure species) was less by from (usually) 9 per cent to 15 per cent than the mass of the yolk of the second egg of the pair (*47*, *p. 330*).

In regard to the first statement, the results of this study show that in the domestic fowl the yolk weight tends to increase slightly up to a production of about 60 eggs, after which it decreases. This is the same general trend followed by egg weight, and since there is no significant difference in the weights of eggs giving rise to females and males, respectively, it does not seem probable that there is any correlation between yolk weight and sex ratio. In regard to Riddle's second statement, it should be observed that the "mass" of yolk in the egg producing a male pigeon is reported to be usually from 9 to 15 per cent less than the "mass" of the yolk producing a female whereas in the domestic fowl there is no such indication of a differential yolk weight situation. Concerning yolk weights in the domestic fowl, Atwood and Weakley (*1*, *p. 35*) make the following observation: "When eggs are laid in cycles, the first egg in the cycle is usually the heaviest, the other eggs decreasing in weight according to their position in the cycle. The weight of the yolk decreases according to the position

of the egg in the cycle." The author (*29*) has shown that in the domestic fowl albumen weight is more highly correlated with egg weight than is yolk weight, while yolk weight is more highly correlated with egg weight than is shell weight. Also, the component parts of the egg contribute in different degrees at different times of the year toward the total egg weight. Increase in the size of yolk apparently is a direct effect of the analogous increase in the size of the body.

Finally, there is reported to be in pigeons a correlation between egg weight and yolk weight, a point which is not adequately demonstrated in the presentation of the material, and there seem to be significant correlations between both egg weight and yolk weight and sex. In the case of the domestic fowl, there is also a correlation between egg weight and yolk weight, in the material of this study found to be $r=0.804\pm0.014$, but there is no correlation between egg weight and sex, as shown in Table VII.

6. THE RELATION OF YOLK WATER CONTENT TO SEX RATIO

In an analysis of all possible factors which might affect the sex ratio, that of the water content of the yolks of eggs might reasonably be supposed to be of minor importance. But since in an extensive series of investigations with another class of birds the factor of yolk water content is assumed to be of considerable significance, it is well to test the application of the theory in the case of the domestic fowl.

TABLE X.—*Showing the relation between yolk water content and sex ratio*

Class	Production	Average weight fresh yolk	Average weight dried yolk	Water	Sex ratio
		Grams	*Grams*	*Per cent*	
1	0-20	16.09	8.21	48.97	62.91±1.44
2	21-40	16.26	8.42	48.15	57.46±1.88
3	41-60	18.32	9.15	50.05	45.00±2.04
4	61-80	19.81	9.88	50.13	44.61±0.69
5	81-100	18.94	9.73	48.62	37.65±1.00
6	101-120	18.91	9.39	50.34	32.20±1.15

With two exceptions, there seems to be a slight increase in the percentage of water contained in the yolks as production increases, but the differences between any two classes are very small indeed and may not be significant. The slightly higher percentages of water content occur during the periods of production when more females than males are produced. But in the case of pigeons the reverse seems to be the case and two comments by Riddle (*46*, *47*) deserve special notice.

> The fifth correlation relates to the percentage of water in eggs of spring and autumn, and in the two eggs of a clutch. These figures for one series of analyses are given on the chart (3) last examined. They show a higher water content for the eggs of the spring (male producers) as compared with the eggs of autumn (female producers); indeed each pair of eggs from the first of the season onward has a slightly higher moisture value than the pair that follows it. The analyses further show a higher percentage of water in the first egg of the clutch than in the second in all cases. If the results of my 800 analyses all ran as smoothly as do the 8 of this series, there would be no doubt of a perfect correlation of high moisture values with small eggs; i. e., with male producing eggs—both small eggs of season and small eggs of individual clutches. But the results are not thus uniform and smooth. There are some series which seem seriously to depart from the order noted above. These can not be discussed here. We can, however, record our own belief that the situation in the chart is, in the main, indicated by the moisture determination. (*46*, *pp. 402-403*).

Chart III, referred to above, is the same as Table VII in the 1917 publication, of which Riddle says (*47*, *p. 336*):

> Two additional methods of determining the amount of water in the yolks, give a satisfactory confirmation of the conclusion that the male-producing ovum contains a higher percentage of water than does the female-producing ovum.

Not only is the degree of correlation between yolk water content and sex not shown, which in 800 analyses should be readily demonstrable, but the records of percentages of water content of yolks are given for one female only. The records as given in Chart III and Table VII show 8 determinations, 2 for the month of May, 1912, 4 for the month of July, 1 for November, and 1 for December. Finally, it would appear that more data are necessary, in the case of the domestic fowl at least, before a correlation may be said to exist between yolk water content and sex ratio.

DISCUSSION

Concerning this study, the situation seems to be as follows: (1) The sex ratio decreases from considerably above fifty in the early period of egg production to considerably below fifty in the advanced period of egg production; (2) there is no indication of reversibility of sex; (3) there is no indication of a modification of the sex ratio through external agencies, such as management and feeding of the laying stock; (4) prenatal mortality does not account for the different sex ratios observed; (5) a differential hatching rate of the sexes does account for the different ratios observed, inasmuch as it has been established that the relative proportion of male-producing to female-producing zygotes changes in relation to antecedent egg production; (6) the most reasonable explanation to account for the changes in the proportion of male-producing to female-producing zygotes seems to be differential maturation of the chromosome complexes.

1. THE SEX CHROMOSOMES IN THE DOMESTIC FOWL

In the case of the domestic fowl Guyer (*21*) described an X chromosome in the male, and in subsequent observations he (*22*) confirmed the original description. Guyer's observations, however, indicate that functionally the male is homozygous, which is in keeping with the apparent homozygosity of the male and the heterozygosity of the female demonstrated by breeding tests, when sex-linked characters are involved. Hance (*24*) has shown that the chromosome number falls within the limits of 30 to 34 and that in the male there are 2 similar chromosomes corresponding in shape and size to the largest unmated chromosome of the female chromosome complex. The results of both Guyer and Hance are in agreement in demonstrating that the male fowl is homozygous and the female heterozygous for sex, as the phenomena of sex-linked inheritance in the domestic fowl demand.

2. SEX RATIO DETERMINATIONS IN THE DOMESTIC FOWL

As a result of this study it has been found that the sex ratio of the domestic fowl, based on families of 10 or more, for the normal hatching season is 48.82 ± 0.80. The sex ratio based upon annual production has been found to be 48.41 ± 0.47, which is in very close agreement with the sex ratio determined for the normal hatching season. But it has also been shown that the first eggs a pullet lays tend to produce a high proportion of males and that as production increases the male ratio decreases.

There are few other statistics demonstrating the sex ratio in the domestic fowl. Darwin (*7, p. 252*) was probably the first to record the proportion of sexes on sufficient numbers to indicate reliability. In 1,001 Cochins reared during eight years by Stretch, as reported by Darwin, the sex ratio was 48.64, but no reference is made as to whether the proportion of sexes as given was determined at hatching time or as the fowls approached maturity. The first edition of the "Descent of Man" was published in 1871, and it is highly improba-

ble that at that time sexes would be determined at hatching time, especially if Stretch bred the buff variety of Cochins, which were so popular in England at that time. Circumstances suggest to the writer that the sex of the Cochins was determined each year as the fowls reached maturity. The next noteworthy record was observed by Field (*14*); in a population of 2,105 individuals the sex ratio proved to be 44.63. Thomsen (*55*) observed a sex ratio of 47.82 in a population of 805 Landhühner and Orpingtons. Finally, Pearl (*44*) in a population of 22,791 chicks, dying in embryo and hatching, of various breeds, using as a basis of determination families of 10 and over, found the sex ratio to be 48.57± 0.28. All of the sex ratios given in this paragraph were obtained on chicks hatched presumably during the normal hatching season.

3. SEX DETERMINATION DURING EMBRYONIC DEVELOPMENT

In the case of birds, where the embryo develops in the egg for some time after the sex is in all probability definitely established, it is obviously difficult to apply methods which might influence the sex after fertilization, even if it were known that such methods were really effective. With the exception of a few experiments on some of the lower animals, "there are very few cases in which there is even a suggestion that the sex of the fertilized egg can be modified by environment, and the belief that this is possible has been entirely abandoned by many of the leading investigators of the subject" (Doncaster, *12*, *p. 147*).

Minoura (*35*) in an interesting study of the effects of gonad grafts in chick embryos, came to the conclusion that sexual differentiation is controllable, to some extent at least, by nongenetic factors. Using the Wyandotte breed of fowl, a small piece of testis or ovary was grafted on the chorio-allantoic membrane of developing embryos varying in age from 2 to 16 days at the time of operation. In a number of embryos between 5 and 13 days of age on which grafts grew and established vascular connections with the allantoic blood vessels, there was a modification of their reproductive systems. The modifications were of such character as to lead Minoura to conclude (*35*, *p. 39*) that "the results demonstrate that the testis and ovary produce secretions which have definite and specific physiological functions and which are capable of modifying the primary sexual characters." That it was the secretions of the gonads which produced the results indicated is established by the fact that grafts of liver, spleen, thyroid, and thymus produced no modification of the reproductive systems in the embryos on which the grafts were made. Minoura concludes, therefore, that sexual differentiation may be reversed in the chick, through the action of sex hormones secreted by the ovary and testis.

> The development and differentiation of one sex is stimulated by the secretion of the gonad of the same sex and inhibited by the secretion of the gonad of the opposite sex. By means of these secretions, the differentiation of sex in the chick can be controlled to some extent. It may further be stated that the two sexes bear a quantitative and not a qualitative relation to each other (*35*, *p. 35*).

Grafts implanted on embryos which had reached a stage of about 5 days' development did not modify the embryos to any marked extent, since up to that stage the allantoic circulation has not been established. Embryos older than about 13 days are not markedly affected, since, as Minoura points out (*35*, *p. 12*), "it is probable that a resistance to foreign tissues has been developed and that such embryos are thereby enabled to destroy the grafts." This is in agreement with Murphy (*41*), who observed that at about the time of hatching a defensive mechanism against foreign tissues develops in the chick. Minoura secured the most marked modifications of the reproductive system in embryos which had reached the eighth and ninth days of development, when the embryo "was in a relatively early stage of sex differentiation." But Swift (*53*, *54*) has

shown that sex is definitely established by the end of the seventh day of incubation. Furthermore, F. R. Lillie (*32*) has discussed quite fully the embryonic development of the ovary and testis through the indifferent period up to the period of sexual differentiation, of which he says (*32, p. 395*): "The sex of the embryo can first be definitely determined about the one hundred and fifty-sixth hour, by the relative sizes of the gonads, by the behavior of the germinal epithelium, and by the presence of a larger number of primordial germ cells in the germinal epithelium of the female." Minoura's statement (*35, p. 34*) that "this period, approximately the second week of incubation, is then the period of active sexual differentiation," does not seem entirely in keeping with Swift's statement (*54, p. 407*) that "at the end of the seventh day of development the sex of the individual can be easily told * * *" It would seem that sexual differentiation has become definitely established by the end of the seventh day of development and that the accounts of subsequent development, as given by F. R. Lillie (*31, 32*), Firket (*15*) and Swift (*53, 54*), involve the further development of the sexual organs as such.

Minoura says that the result of his experiments shows "that sexual differentiation may be reversed in the chick. This result supports the explanation advanced by Lillie to account for the production of the freemartin in cattle." But in regard to the freemartin situation in cattle, F. R. Lillie and Bascom (*34*) say that—

> In the female of cattle sex differentiation before birth is apparently due to genetic factors exclusively; in the male the genetic factors are intensified by the production of a hormone.

This situation in cattle as reported by F. R. Lillie and Bascom is similar to that in the domestic fowl as reported by Boring and Pearl (*3*), Morgan (*39, 40*), Goodale (*19, 20*), and Cole and Lippincott (*4*). The results of these investigators all go to show that the relation between the gonads and the secondary sexual characters is specific and not general. The results have demonstrated the existence of a mechanism for the control of the secondary sexual characters that is so closely associated with certain parts of the mechanism for the determination of sex that the two go together. Hormones or chalones are secreted (Boring and Pearl *2*, Goodale *20*, and Morgan *40*) which affect the development of the secondary sexual characters, and it would have proven very interesting in this connection if Minoura had grafted pieces of testis from Brown Leghorns on the developing embryos of Barred Plymouth Rocks and reared the chicks to maturity. A reversibility of sex, as claimed by Minoura, would be exhibited if there appeared modifications of the normal sex-linked situation in the cross between Brown Leghorn males and Barred Plymouth Rock females. This is the cross that was made in the present study, and apparently there was no tendency toward reversibility of sex, in spite of the variation in sex ratio in relation to egg production.

The same objection may be offered with reference to Riddle's claims of sex reversibility in the case of pigeons. Riddle (*46, p. 410*) claims to have demonstrated that "germs normally female producing, have, under experiment, been made to develop males; and that germs which were prospectively male producing have been made to form female adults." But Sturtevant (*52*), in connection with a particular cross in which Riddle claimed to have secured an excess of female doves due to "changing over" of the males, has suggested that the probable explanation rests in the selective elimination of male-producing chromosomes during the maturation process. Furthermore, in the present study an excess of females during the periods of increased egg production is certainly not due to "changing over" of males, there being no indication whatever of a modification of the characteristic plumage color of each sex in the chicks; the plumage color

is always inherited in a definite sex-linked Mendelian manner. Also, only in very few cases did there appear to be an abnormal condition of the gonads at the time of dissection.

Two cases concerning assumed reversibility of sex have been reported recently, Crew (*5*) in the case of a Buff Orpington chicken and Riddle (*48*) in the case of a pigeon. The case reported by Crew deserves special mention, inasmuch as he placed undoubted reliance upon the statements of the original owner of the chicken concerning its apparent sex up to three and one-half years of age. The chicken is stated by Crew to have been "a purebred Buff Orpington, a good layer and a mother of chickens," although Crew makes no mention of the bird laying eggs after he secured it. It is very unfortunate, therefore, that Crew was not able to secure undisputed evidence concerning the original sex of the chicken.

Suppose, however, that the chicken was originally a female with a sex chromosome constitution of XY. Suppose also that sex reversal actually occurred, spermatozoa of XY constitution being produced, as Crew suggests. Such a bird when mated to a normal female should give a sex ratio of 33.33, since the YY chromosome complex constitution would not develop. Now, if the normal female had possessed the sex-linked barring factor, then half of the female progeny should have been black and half barred instead of all being black, as in the case of the present study. Crew was able to raise only two chickens from the mating, one male and one female, numbers too small to indicate the sex chromosome constitution of the chicken for which it is claimed the sex became reversed.

The results of the present study involving several matings and several hundred chicks from mothers possessing the sex-linked barring factor have provided no evidence of change in sex during embryonic development. Cases of reputed sex reversibility where all of the evidence may be unquestioned concerning the sex of the individual from hatching time to the time sex is supposed to have been reversed are awaited with considerable interest.

Finally, it may be said that it is possible that the sex of an embryo may be modified after fertilization if the physiological condition on which sex depends can be changed. Based upon the results of the present study the statement may be made that until more convincing evidence is available it seems necessary to conclude that sex determination is affected only at or prior to fertilization.

4. SEX DETERMINATION AT FERTILIZATION

The process of fertilization is uniform in all its essentials throughout the animal kingdom. It consists in the union of two nuclei derived usually from two parents, with the mingling and division of their substance "in such a way that the chromosomes from each parent are equally represented in both the daughter nuclei produced by the first segmentation division" (Doncaster, *13*, *p. 117*). The process of fertilization serves as about the only definite indication of the nature and function of sex. Since there are only two distinct sexes and since in any species the average ratio in which they are produced is approximately constant, it is obvious that the mechanism for producing the equal division of chromosomes is of fundamental significance. Jennings (*27*, *p. 193*) has stated the problem simply and clearly: "The really fundamental thing that mating does is *to produce new combinations of hereditary characters.*"

The statement made above, concerning the equal representation of the chromosomes in the daughter nuclei, is of a general character, and does not apply to those cases where one sex is homozygous and the other heterozygous for the sex character. In the case of the domestic fowl, it has been shown previously from cytological observations that the female is heterozygous and the male homozygous for sex. This fact is amply supported by the breeding results secured by

a number of workers, Davenport (*8, 9, 10, 11*), Goodale (*16, 17, 18*), Hadley (*23*), Pearl and Surface (*42, 43*), Morgan and Goodale (*38*), Spillman (*50*), and Sturtevant (*51*). The results of these experiments involving the inheritance of sex-linked characters are of common knowledge, and their significance from the standpoint of the Mendelian inheritance of sex is so well established that nothing further regarding them will be said for the present.

It has been quite clearly established, therefore, that in the case of the domestic fowl sex is inherited, under normal conditions, as a Mendelian character. Under such conditions chance combination of the germ cells produces equal numbers of homozygotes and heterozygotes, which fact explains the approximate equality of the sexes. Sex is apparently determined at the time of fertilization and is associated with the presence or absence of a second sex chromosome.

It has been shown in this study that as egg production increases the sex ratio decreases. Pearl (*45*) made a similar observation as a result of an extensive study of the relation between egg production and the sex ratio during the normal hatching season. It was found that "the larger the number of eggs which a hen lays before being put into the breeding pen, the larger will be the proportion of females and the smaller the proportion of males produced by her eggs." It is regrettable that shortly after the publication of the brief note concerning the nature of his results, all of Pearl's material bearing on this problem was lost through an unfortunate accident. Apparently the decrease of the sex ratio in the present study can not be accounted for on the basis of prenatal mortality, for it has been shown that prenatal mortality affects the ratio to a slight extent only, and to the same extent throughout the year.

What factors are operative, therefore, in effecting an alteration in the sex ratio from an approximate equality of sexes which has been found to exist during the normal hatching season? There is a differential hatching rate of the sexes between the period of early egg production and the period of later production, as shown in Table III. Apparently the difference in the proportion of sexes produced from one period of egg production to another is accounted for by the difference in the absolute numbers of sexes produced from one period of egg production to another. Since in the domestic fowl the male is homozygous for sex, it does not seem probable that the spermatozoa would influence the situation, at least not from the standpoint of affecting the mechanism of the fertilization process. The fertilization process is a reaction process possessing very definite biological and biochemical characteristics, as pointed out by F. R. Lillie (*33*), and although the spermatozoa may provide a stimulus while the egg is still in the second maturation stage, there is no known reason why the spermatozoa might affect a change in sex determination from one period of egg production to another. Bearing on this point, an interesting case has been reported by Hays (*25*) where the amount of sexual service in male rabbits, which are heterozygous for the sex character, affects the sex ratio, there being a predominance of males in the first service group and then usually an increasing predominance of females as the number of services is increased. Hays has mentioned two possibilities to account for this situation:

> Either female-producing spermatozoa are formed more largely than male-producing spermatozoa as the amount of service of the male increases, or the male-producing sperm are in themselves weaker than the female-producing sperm and consequently fewer of them survive to take part in fertilization.

There is no evidence on the first point, and in connection with the second point it is suggested that the female-producing sperm, because of its larger size and greater chromatin content, may be more vigorous than the male-producing sperm and thus more apt to first reach the egg for the process of fertilization. According to unpublished results of Lush, however, there is no apparent differ-

ence in size between male and female producing spermatozoa in the rabbit. The explanation offered will not hold for the domestic fowl, however, where the male is homozygous for the sex character. If there is selective fertilization, from the standpoint of male-producing or female-producing eggs being more readily fertilized under certain conditions, the only tenable explanation would seem to lie in some causes affecting the maturation of the egg.

5. SEX DETERMINATION PRIOR TO FERTILIZATION

Apparently a solution of the problem concerning modifications in the normal sex ratio in the domestic fowl belongs to the domain of gamete development. It has been shown that sex is apparently not reversible during embryonic development and, furthermore, that sex is probably determined at the time of fertilization. Deviations from approximate equality of the number of males and females must be interpreted in terms of influences affecting the development of the gametes which subsequently take part in the process of fertilization.

Along this line must first be mentioned the possibility of differential maturation, since it has already been observed that during the early period of egg production the actual number of male-producing zygotes exceeds the actual number of female-producing zygotes, and that as egg production increases the situation tends to be reversed. The possibility of the existence of differential maturation has been shown to exist in some forms of insects. In the case of the currant moth, *Abraxas grossulariata*, Doncaster (*12*) bred seven generations of a strain of Abraxas in which some broods in each generation were all females. Doncaster (*12, p. 132*) suggests the possibility that "in these families all of the eggs have a tendency to extrude the male-determining sex chromosome, just as in aphids the male-producing eggs all extrude the female-producing X chromosome." In *Talaeoporia tubulosa*, Seiler (*49*) has shown that in the anaphase of the first polar division the unpaired X chromosome seems to go into the polar body rather more frequently than into the oocyte nucleus. In this species females are more numerous than males, so that genetic and cytological facts are in agreement.

Might a simliar condition exist in the case of the domestic fowl? The process of maturation begins in the fully ripe follicle and is completed after ovulation. At the time of ovulation the first maturation spindle is still in the equatorial plate stage, the outer end of the spindle being in almost immediate contact with the surface of the ovum. The first polar body is thrown off and almost immediately the second maturation spindle is formed. The position of the second maturation spindle is similar to that of the first, and a second polar body is thrown off. Whichever maturation division serves as the reduction division must also serve in the elimination of a greater number of female-producing than male-producing complexes during the early period of egg production and the elimination of a greater number of male-producing than female-producing complexes during the later periods of egg production, if this is the explanation of the results obtained. During the normal hatching season there would seem to be no influence at work affecting the apparent chance elimination of either kind of complex. Is there any evidence concerning the selective elimination of complexes of a particular kind? Heape (*26*) has thus interpreted his results with canaries bred under different conditions. He found that certain conditions affected the sex ratio, and he assumed that under conditions of early breeding and light feeding male-producing gametes are produced in excess, while under conditions of late breeding and heavy feeding female-producing gametes are produced in excess. Heape's results are similar in nature to those secured by

Pearl (*45*), Riddle (*46*, *47*), Crew and Huxley (*6*), and the results secured in this study, except that in the last four cases the method of feeding has apparently had no influence, inasmuch as the birds were all fed similarly throughout the duration of the tests. In the case of the present study, then, if differential maturation occurred it must be accounted for as a result of conditions of egg production. That is, differential maturation must be looked upon as an effect, produced by certain conditions, the nature of which will be discussed presently.

A very interesting case, bearing on the discussion as it has been developed up to the present, is reported in one of the forms closely related to the plant lice *Phylloxera fallax*. Morgan (*36*, *37*) has shown that the sex of the individual developing from the parthenogenetic egg is determined before the distribution of sex chromosomes at polar body formation. This is explained by the fact that the male-producing and female-producing eggs are already differentiated from one another before the time of polar body formation. The eggs which will develop into males through the elimination of the double sex chromosome are smaller than the female-producing eggs, where there is no elimination. In this case, therefore, the elimination of the sex chromosome is not a matter of chance but is determined by conditions antecedent to the time of polar-body formation. Such conditions, therefore, constitute a sex-determining factor in the chain of causation of sex.

So it would seem to be in the case of the domestic fowl. As to the nature of these conditions, hardly more than speculative suggestions can be offered, but perhaps they will not be less speculative than other theories advanced to account for the manner in which sex is determined.

It would seem that many of the more general statements implying that sex determination is readily modified by external conditions and that sex may be controlled are not in harmony with all the facts. The approximate equality of the sexes is one of the outstanding phenomena among the higher forms which reproduce bisexually. But if environmental factors play such a highly significant part in the modification of sex determination as is so frequently claimed, is it not almost inconceivable that with so many organisms under such varying environmental conditions throughout all parts of the world the sexes should usually approximate equality? Furthermore, it may be legitimate to inquire as to the nature of sex potencies which are assumed to be operative in producing sex and what causes contribute to the development of different degrees of potency. It is now apparent that in some of the experiments designed to test the stability of the sex-determining mechanism many of the interpretations based upon the results secured require examination.

A number of factors have been found to be associated with one sex or the other, and the results secured in several lines of work would seem to be materially weakened by the apparent desire to disregard, in a large measure, the significance of the chromosomal mechanism, which is continually receiving stronger and stronger support from cytological investigations. It should not be overlooked that notwithstanding the laborious procedure and technical difficulties involved in conducting cytological investigations remarkable achievements have been accomplished in definitely correlating cytological facts with genetic results for an ever increasing number of forms. If the different phenomena which have been found to be associated with one sex or the other can also be shown to be associated with the constitution of the chromosome complex then it would appear that the metabolic and chromosome theories can be harmonized and real progress made toward a solution of the problem of the causation of sex.

As far as the chromosome theory of sex is concerned, in view of the variety of circumstances under which sex ratios have been modified and the mode of sex

determination apparently affected, and also in view of the most recent interpretations concerning the physiological constitution of the chromosomes, with their associated interactions, it seems certain that the simple hypothesis of an unchangeable hereditary entity must be modified. Two views may be expressed as follows: The sex chromosome may cause sex, or the presence or absence of the sex chromosome may be the effect of the two sexes being produced by differing levels of metabolism. At the same time, in the case of the domestic fowl, as in many of the other higher forms, the presence or absence of a second sex chromosome seems to be definitely associated with sex. But it is evident that sex can not depend on a chromosome alone, for the chromosome obviously acts by its relation with the cell protoplasm. Seemingly the presence of a particular chromosome provides only one side of a reciprocal reaction, while the determination of sex must depend upon the complete result of all the reciprocal reactions.

There may not be any heredity other than Mendelian, and although it is evident that the chromosomes are responsible for the segregation of Mendelian characters, it has become apparent that the cytoplasm can not be relegated to a position of serving as a medium only in which the chromosomes carry on their reactions. The chromosomes can not be regarded as acting independently of the cytoplasm and in fact there is much evidence of interaction between chromosomes and cytoplasm. The coexistence in the primary oocyte of the germinal vesicle and the enormous growth of the cell with its formation of reserve food material suggests a causal connection between them. The diffusion of chromatin in the ordinary resting nucleus has the result of increasing its area in proportion to its mass and thus favors active metabolism. Similarly, it has been held that the excessive diffusion of chromosomes in the germinal vesicles of many animals gives rise to an intense activity of the chromosomes in the elaboration of yolk. Finally, it may be said that the interaction which takes place between nucleus and cytoplasm is certain to effect a modification in both nucleus and cytoplasm.

Applying these ideas to the case in hand, it does not seem unreasonable to suppose that the nature of the reactions within the nucleus would vary from time to time throughout the maturation process. Variations in the chemical composition of the eggs in relation to increased egg production in the domestic fowl which have been shown to exist, might also exert an influence on the nature of interactions between the chromosomes and the cytoplasm. It is quite conceivable, therefore, that during the early period of egg production a given condition of reactions might prevail, and this particular condition might be modified as egg production increased. This being possible, it is also possible that under a given set of conditions certain factors may operate in causing the female-producing complex to be extruded more frequently in polar-body formation than the male-producing complex, and conversely when the conditions become altered. If this be possible, then it is reasonable to suppose that during the early period of egg production in the domestic fowl female-producing complexes are extruded more frequently than male-producing complexes and that as production increases the situation becomes reversed. Finally, since it seems obvious that there are more male-producing than female-producing zygotes produced during the early period of egg production and that this situation becomes reversed as egg production increases, and since it therefore follows that there must, of necessity, be differential maturation, this hypothesis has been suggested to account for more males than females being produced during the early period of egg production and more females than males during the later period of egg production.

SUMMARY

1. In the case of the domestic fowl, there is found to be a significant negative correlation between antecedent egg production and the sex ratio.

2. The sex ratio of the three yearly populations is 48.41 ± 0.47.

3. The sex ratio, based on families of 10 or more, for the normal hatching season, is 48.82 ± 0.80.

4. With the annual egg production divided into classes of 20 eggs each, the sex ratio, based upon antecedent production, is as follows: 0–20, 62.91 ± 1.44; 21–40, 57.46 ± 1.88; 41–60, 45.00 ± 2.04; 61–80, 44.61 ± 0.69; 81–100, 37.65 ± 1.00; 101–120, 32.20 ± 1.15.

5. The correlation between antecedent egg production and sex ratio is -0.704 ± 0.037.

6. The correlation between season and sex ratio is -0.165 ± 0.076, and is probably incidental.

7. There is no apparent correlation between egg weight and sex ratio.

8. There is no apparent correlation between yolk weight and sex ratio.

9. There is no apparent correlation between yolk water content and sex ratio.

10. The feeding and general management of the birds having been practically identical for the three years, it is obvious that general environmental conditions have not affected the sex ratio.

11. The variation in the sex ratio in relation to egg production can not be accounted for on the basis of prenatal mortality.

12. There is no indication of reversibility of sex, this possibility having been tested by using the dominant sex-linked barring character of Barred Plymouth Rocks with the recessive plumage color character of Brown Leghorns.

13. It is apparent that male-producing zygotes are produced in excess of female-producing zygotes during the early period of egg production and that as egg production increases female-producing zygotes tend to be produced in excess.

14. An hypothesis is developed to account for differential maturation, which in turn would account for the modified sex ratios.

LITERATURE CITED

(1) Atwood, H., and Weakley, C. E., Jr.
1917. CERTAIN CHARACTERISTICS OF HEN EGGS. W. Va. Agr. Exp. Sta. Bul. 166, 35 p.

(2) Boring, A. M., and Pearl, R.
1917. SEX STUDIES. IX. INTERSTITIAL CELLS IN THE REPRODUCTIVE ORGANS OF THE CHICKEN. Anat. Rec. 13: 253–268, illus.

(3) ——— ———
1918. SEX STUDIES. XI. HERMAPHRODITE BIRDS. Jour. Exp. Zool. 25: 1–47, illus.

(4) Cole, L. J., and Lippincott, W. A.
1919. THE RELATION OF PLUMAGE TO OVARIAN CONDITION IN A BARRED PLYMOUTH ROCK PULLET. Biol. Bul. 36: 167–182, illus.

(5) Crew, F. A. E.
1923. STUDIES IN INTERSEXUALITY. II. SEX-REVERSAL IN THE FOWL. Proc. Roy. Soc. [London] (B) 95: 256–278, illus.

(6) ——— and Huxley, J. S.
1923. THE RELATION OF INTERNAL SECRETION TO REPRODUCTION AND GROWTH IN THE DOMESTIC FOWL. 1. EFFECT OF THYROID FEEDING ON GROWTH RATE, FEATHERING, AND EGG PRODUCTION. Vet. Jour. 79: 343–348.

(7) DARWIN, C. R.
1875. THE DESCENT OF MAN. Rev. ed. 688 p., illus. New York.
(8) DAVENPORT, C. B.
1906. INHERITANCE IN POULTRY. 136 p., illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 52.)
(9) ———
1909. INHERITANCE OF CHARACTERISTICS IN DOMESTIC FOWL. 100 p., illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 121.)
(10) ———
1911. ANOTHER CASE OF SEX-LIMITED HEREDITY IN POULTRY. Proc. Soc. Exp. Biol. & Med. 9: 19–20.
(11) ———
1912. SEX-LIMITED INHERITANCE IN POULTRY. Jour. Exp. Zool. 13: 1–26, illus.
(12) DONCASTER, L.
1914. THE DETERMINATION OF SEX. 172 p., illus. Cambridge.
(13) ———
1920. AN INTRODUCTION TO THE STUDY OF CYTOLOGY. 280 p., illus. Cambridge.
(14) FIELD, G. W.
1901. EXPERIMENTS ON MODIFYING THE NORMAL PROPORTION OF THE SEXES IN THE DOMESTIC FOWL. Biol. Bul. 2: 360–361.
(15) FIRKET, J.
1914. RECHERCHES SUR L'ORGANOGENÈSE DES GLANDES SEXUELLES CHEZ LES OISEAUX. Arch. Biol. 29: 201–351, illus.
(16) GOODALE, H. D.
1909. SEX AND ITS RELATION TO THE BARRING FACTOR IN POULTRY. Science 29: 1004–1005.
(17) ———
1910. BREEDING EXPERIMENTS IN POULTRY. Proc. Soc. Exp. Biol. & Med. 7: 178–180.
(18) ———
1911. SEX-LIMITED INHERITANCE AND SEXUAL DIMORPHISM IN POULTRY. Science 33: 939–940.
(19) ———
1916. GONADECTOMY IN RELATION TO THE SECONDARY SEXUAL CHARACTERS OF SOME BIRDS. 52 p., illus. Washington. D. C. (Carnegie Inst. Wash. Pub. 243.)
(20) ———
1919. INTERSTITIAL CELLS IN THE GONADS OF DOMESTIC FOWL. Anat. Rec. 16: 247–250, illus.
(21) GUYER, M. F.
1909. THE SPERMATOGENESIS OF THE DOMESTIC CHICKEN. (Gallus gallus dom.) Anat. Anz. 34: 573–580, illus.
(22) ———
1916. STUDIES ON THE CHROMOSOMES OF THE COMMON FOWL AS SEEN IN TESTES AND IN EMBRYOS. Biol. Bul. 31: 221–268, illus.
(23) HADLEY, P. B.
1910. SEX-LIMITED INHERITANCE. Science 32: 797.
(24) HANCE, R. T.
1923. CHICK CHROMOSOMES AND SEX DETERMINATION. (Abstract.) Anat. Rec. 26: 348–349.

(25) HAYS, F. A.
1918. THE INFLUENCE OF EXCESSIVE SEXUAL ACTIVITY OF MALE RABBITS. Jour. Exp. Zool. 25: 571–613, illus.
(26) HEAPE, W.
1907. NOTE ON THE INFLUENCE OF EXTRANEOUS FORCES UPON THE PROPORTION OF THE SEXES PRODUCED BY CANARIES. Proc. Cambridge Phil. Soc. 14: 201–205.
(27) JENNINGS, H. S.
1920. LIFE AND DEATH, HEREDITY AND EVOLUTION IN UNICELLULAR ORGANISMS. 233 p., illus. Boston.
(28) JULL, M. A.
1923. DIFFERENTIAL SEX GROWTH CURVES IN BARRED PLYMOUTH ROCK CHICKS. Sci. Agr. 4: 58–65, illus.
(29) ———
1924. EGG WEIGHT IN RELATION TO PRODUCTION. I. THE RELATIONSHIP OF THE WEIGHTS OF THE PARTS OF THE EGG TO THE TOTAL EGG WEIGHT. Poultry Sci. 3: 77–88.
(30) ———
1924. EGG WEIGHT IN RELATION TO PRODUCTION. II. THE NATURE AND CAUSES OF CHANGES IN EGG WEIGHT IN RELATION TO ANNUAL PRODUCTION IN PULLETS. Poultry Sci. 3: 153–170.
(31) LILLIE, F. R.
1908. THE DEVELOPMENT OF THE CHICK. 472 p., illus. New York.
(32) ———
1919. THE DEVELOPMENT OF THE CHICK. Ed. 2, rev. 472 p., illus. New York.
(33) ———
1919. PROBLEMS OF FERTILIZATION. 278 p., illus. Chicago.
(34) ——— and BASCOM, K. F.
1922. AN EARLY STAGE OF THE FREE-MARTIN AND THE PARALLEL HISTORY OF THE INTERSTITIAL CELLS. Science 55: 624–625.
(35) MINOURA, T.
1921. A STUDY OF TESTIS AND OVARY GRAFTS ON THE HEN'S EGG AND THEIR EFFECTS ON THE EMBRYO. Jour. Exp. Zool. 33: 1–62, illus.
(36) MORGAN, T. H.
1909. A BIOLOGICAL AND CYTOLOGICAL STUDY OF SEX-DETERMINATION IN PHYLLOXERANS AND APHIDS. Jour. Exp. Zool. 7: 239–352, illus.
(37) ———
1909. SEX DETERMINATION AND PARTHENOGENESIS IN PHYLLOXERANS AND APHIDS. Science 29: 234–237.
(38) ——— and GOODALE, H. D.
1912. SEX-LINKED INHERITANCE IN POULTRY. Ann. N. Y. Acad. Sci. 22: 113–133, illus.
(39) ———
1919. THE GENETIC AND THE OPERATIVE EVIDENCE RELATING TO SECONDARY SEXUAL CHARACTERS. 108 p., illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 285).
(40) ———
1920. THE EFFECTS OF CASTRATION OF HEN FEATHERED CAMPINES. Biol. Bul. 39: 231–247, illus.
(41) MURPHY, J. B.
1914. STUDIES IN TISSUE SPECIFICITY. III. FACTORS OF RESISTANCE TO HETEROPLASTIC TISSUE-GRAFTING. Jour. Exp. Med. 19: 513–522, illus.

(42) PEARL, R., and SURFACE, F. M.
1910. ON THE INHERITANCE OF THE BARRED COLOR PATTERN IN POULTRY. Arch. Entwick. Organism 30: 45–61, illus.

(43) ——— ———
1910. FURTHER DATA REGARDING THE SEX-LIMITED INHERITANCE OF THE BARRED COLOR PATTERN IN POULTRY. Science 32: 870–874.

(44) ———
1917. THE SEX RATIO IN THE DOMESTIC FOWL. Proc. Amer. Phil. Soc. 56: 416–436, illus.

(45) ———
1917. FACTORS INFLUENCING THE SEX RATIO IN THE DOMESTIC FOWL. (Abstract) Science 46: 220.

(46) RIDDLE, O.
1916. SEX CONTROL AND KNOWN CORRELATIONS IN PIGEONS. Amer. Nat. 50: 385–410.

(47) ———
1917. THE CONTROL OF THE SEX RATIO. Jour. Wash. Acad, Sci. 7: 319–356, illus.

(48) ———
1924. A CASE OF COMPLETE SEX-REVERSAL IN THE ADULT PIGEON. Amer. Nat. 58: 167–181, illus.

(49) SEILER, J.
1917. GESCHLECHTSCHROMOSOMEN UNTERSUCHUNGEN AN PSYCHIDEN. Ztschr. Indukt. Abstam. 18: 81–92, illus.

(50) SPILLMAN, W. J.
1908. SPURIOUS ALLELOMORPHISM: RESULTS OF SOME RECENT INVESTIGATIONS. Amer. Nat. 42: 610–615.

(51) STURTEVANT, A. H.
1911. ANOTHER SEX-LIMITED CHARACTER IN FOWLS. Science 33: 337–338.

(52) ———
1920. INTERSEXES IN DROSOPHILA SIMULANS. Science 51: 325–327.

(53) SWIFT, C. H.
1915. ORIGIN OF THE DEFINITIVE SEX-CELLS IN THE FEMALE CHICK AND THEIR RELATION TO THE PRIMORDIAL GERM-CELLS. Amer. Jour. Anat. 18: 441–470, illus.

(54) ———
1915. ORIGIN OF THE SEX-CORDS AND DEFINITIVE SPERMATOGONIA IN THE MALE CHICK. Amer. Jour. Anat. 20: 375–410, illus.

(55) THOMSEN, E.
1911. DIE DIFFERENZIERING DES GESCHLECHTS UND DAS VERHÄLTNIS DER GESCHLECHTER BEIM HÜHNCHEN. Arch. Entwick. Organism 31: 512–530, illus.

A GRADIENT OF PERMEABILITY TO IODIN IN WHEAT SEED COATS[1]

(A PRELIMINARY NOTE)

By HARRY BRAUN[2]

Assistant Pathologist, Laboratory of Plant Pathology, Bureau of Plant Industry, United States Department of Agriculture

Statements in literature on the relation of semipermeable seed coats to the entrance of toxic solutes, expressed as recently as 1923 (Harrington and Crocker),[3] are to the effect that a solute like iodin enters only to a small degree through the general surface of the grain, but largely through the hilum or micropyle whence it spreads laterally and in a distal direction under the seed coats. Experiments by the writer point to contrary conclusions as follows: (1) there is very little spread of iodin under the seed coats from the embryo end either laterally or in a distal direction; (2) this is equally true for all other parts of the seed; (3) entrance of iodin takes place over the entire surface of the grain radially but not uniformly; (4) the *apparent* lateral and distal spread from the embryo end is due to the existence of a gradient of permeability to iodin in the seed coats, which permeability is greatest near the embryo end, diminishes to a minimum near the distal end and again increases slightly. These conclusions are supported by experiments with wheat seeds coated in various ways with waterproof substances like paraffin. The experiments have shown that (1) when the embryo end only is exposed, iodin penetrates only as far as the limits of the exposed area and fails to spread much further laterally or distally under the seed coats protected by paraffin; (2) the same is true for any exposed region of the kernel partially paraffin-coated, the stain only goes as far as the limits of the exposed part; (3) when the embryo end alone is coated, the entrance of iodin manifests itself progressively, beginning always on the exposed part nearest the embryo end 180 degrees from the grooved surface and continuing in a lateral and distal direction but not toward the coated embryo end; and the blackening due to iodin at any point on such a grain corresponds in degree with that shown after a similar period of time on the same point of a wholly unprotected grain.

Ultimately, the entire grain is softened by entrance and spread of water from the iodin solution but is not discolored except near the exposed surface. This indicates that the failure of iodin to spread rapidly laterally and inwardly is due to its removal either (1) by chemical combination with the starch nearest the point of entry, or (2) by adsorption (Biltz)[4] on the starch immediately underneath the exposed area, while water goes through.

---

[1] Received for publication Jan. 23, 1924. Presented at the Cincinnati meeting, A. A. A. S., Dec. 31, 1923, before Physiology Section, Botanical Society of America.

[2] The writer is deeply indebted to Prof. R. A. Harper, of Columbia University, in whose laboratory the greater part of the experiments were made.

[3] HARRINGTON, G. T., and CROCKER, W.—STRUCTURE, PHYSICAL CHARACTERISTICS, AND COMPOSITION OF THE PERICARP AND INTEGUMENT OF JOHNSON GRASS SEED IN RELATION TO ITS PHYSIOLOGY. Jour. Agr Research 23: 193-222, illus. 1923.

[4] BILTZ, W.—UEBER DIE BLAUE ADSORPTIONSVERBINDUNG VON BASISCHEM LANTHANACETAT UND [illegible] Ber. Deut. Chem. Gesell. 37: 719-724. 1904.

The removal of iodin by the nearest starch layer was corroborated by experiments with three-eighths inch cubes of dry starch gels, placed in iodin solution. These became completely soft through entrance of water but remained colorless except for a blackened surface layer which was narrow and fairly well delimited in cross-section.

Microtome sections of immature wheat seeds fixed in Flemming's strong solution showed near the distal end a deep blackening of the cutin layer (more strongly cutinized in the mature seed) with scarcely any discoloration at the embryo end, and a gradation of color between. The greater degree of cutinization first demonstrated by osmic acid in a direction opposite to the permeability to iodin was subsequently found in the mature seed by the use of Flemming's triple stain and this makes it certain that the cutinization gradient remains nearly the same in the mature seed, although this may be obscured owing to the higher degree of cutinization attained in the entire surface layer. This indicates a cytological basis for the gradient of permeability experimentally observed.

FURTHER STUDIES ON THE RELATIVE SUSCEPTIBILITY TO CITRUS CANKER OF DIFFERENT SPECIES AND HYBRIDS OF THE GENUS CITRUS, INCLUDING THE WILD RELATIVES [1]

By GEORGE L. PELTIER, *formerly Plant Pathologist, Alabama Agricultural Experiment Station, and Agent, Bureau of Plant Industry, United States Department of Agriculture,* and WILLIAM J. FREDERICH, *formerly Assistant Pathologist, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The object of this paper is to present in a summarized form the results of the writer's investigations, extending from 1917 to 1921, on the relative susceptibility of citrus canker of a large number of different species, hybrids, and relatives of the genus Citrus. During the progress of this investigation two detailed reports [2,3] have appeared. Since the last report was issued, new plants have been added to the experiments, further numbers successfully infected and in some cases continued observations have modified previous conclusions.

The reader is referred to the reports already published for the description of the plants used, the location of the experiments, experimental methods employed and the detailed observations made. The complete data on each plant included in the experiments during the course of the investigation are on file in the Office of Crop Physiology and Breeding Investigations, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

SUSCEPTIBILITY OF THE WILD RELATIVES OF THE GENUS CITRUS

Repeated attempts to infect *Melia azedarach* L., in the field were unsuccessful. Likewise, under greenhouse conditions, negative results were obtained with *Xanthoxylum bungei* Planch., Xanthoxylum sp., *Toddalia asiatica* (L.) Kurz and *Glycosmis pentaphylla* Correa. A few small infections on leaves were obtained under greenhouse conditions with *Claucena lansium* (Lour.) Skeels, and *Chalcas exotica* (L.) Millsp. However, these results could not be duplicated in the field. *Casimiroa edulis* Lav. & Lex., was the only plant of this group that was successfully infected at wounds on the leaves, in the field and greenhouse.

[1] Received for publication Feb. 16, 1924. Published with the approval of the Director of the Alabama Agricultural Experiment Station. The paper is based on cooperative investigations between the Office of Crop Physiology and Breeding Investigations, Bureau of Plant Industry, U. S. Department of Agriculture and the Department of Plant Pathology, Alabama Agricultural Experiment Station.

[2] PELTIER, G. L. SUSCEPTIBILITY AND RESISTANCE TO CITRUS-CANKER OF THE WILD RELATIVES, CITRUS FRUITS, AND HYBRIDS OF THE GENUS CITRUS. [Preliminary paper.] Jour. Agr. Research 14: 337-358, illus. 1918.

[3] ——— and FREDERICH, W. J. RELATIVE SUSCEPTIBILITY TO CITRUS-CANKER OF DIFFERENT SPECIES AND HYBRIDS OF THE GENUS CITRUS, INCLUDING THE WILD RELATIVES. Jour. Agr. Research 19: 339-362, illus. 1920.

Chaetospermum glutinosum (Blanco) Swingle was quite easily infected both in the greenhouse and field in the absence of wounds. In fact, natural infection from adjoining plants in the field frequently occurred. Judging from its susceptibility under southern Alabama conditions, it is as susceptible as some of the Citrus fruits. However, these plants can not grow in southern Alabama as they are very easily killed by the winter temperatures generally prevailing in this region. Of the remaining plants belonging to the subtribe Aeglinae, *Balsamocitrus dawei* Stapf and *Aeglopsis chevalieri* Swingle remained free from infection after repeated attempts to infect, while *Aegle marmelos* Correa, although infected at wounds on the leaves in the greenhouse, remained nonsusceptible in the field.

Feronia limonia (Correa) Swingle was infected to some extent at wounds in the greenhouse. No results were obtained in the field. In the case of *Feroniella lucida* Swingle, however, positive results were obtained both in the greenhouse and field.

On the plants in subtribe Lavanginae, two species, *Triphasia trifolia* (Burm.) P. Wilson and *Severinia buxifolia* Ten. proved to be strictly nonsusceptible in the field and greenhouse, while *Paramignya monophylla* Wight was easily infected in the greenhouse. Likewise, plants of *Hesperethusa crenulata* (Roxb.) Roem. were easily infected in the greenhouse and field through natural infection from adjoining plants.

In the case of *Severinia buxifolia* it was repeatedly observed that the leaves, petioles, and stems when punctured or wounded had the ability to form cork tissues very rapidly. Owing to the nonsusceptibility of *Severinia buxifolia* to citrus canker and the fact that it thrives under southern Alabama conditions, its propagation as a hedge plant to replace *Poncirus trifoliata* (L.) Raf. should be encouraged. Incidentally, it may prove suitable as a stock for some types of Citrus fruits.

All plants of the subtribe Citrinae were successfully infected, but in varying degrees. Attempts to infect *Citropsis schweinfurthii* Swingle and M. Kellerman in the field were unsuccessful, although small unruptured spots were produced at wounds in the greenhouse. The two species of Atalantia have been rather easily infected. Likewise, plants of *Eremocitrus glauca* (Lindl.) Swingle were quite easily infected both in the greenhouse and field.

The three species and one variety of Microcitrus and *Eremocitrus glauca* have proved susceptible enough under controlled conditions to be naturally infected in their native habitat, if a source for infection was present. Both of these genera are native of the east coast of Australia, and while citrus canker has only been reported from the northern territory of Australia, should canker be introduced at any time into the Citrus districts of Queensland, there is a bare possibility that citrus canker might be disseminated to the native growths of Microcitrus and Eremocitrus.

Fortunella hindsii (Oliver) Swingle is rather susceptible; much more so than any of the other three species of kumquats. While these were all successfully infected in the greenhouse, only two, *F. japonica* (Thunb.) Swingle and *F. Margarita* (Thunb.) Swingle gave positive results in the field.

In the spring of 1918, thirty 3-year-old trees of *Fortunella margarita* budded on *Poncirus trifoliata* were planted in the isolation field. From the summer of 1918 through the season of 1921, not a single canker spot was found on these trees, notwithstanding the fact that various methods of inoculations were tried and that during the active growing season they were at all times exposed to natural infection. The kumquats, with the exception of *F. hindsii* are highly resistant to Citrus canker and it is only under the most favorable field conditions and through wounds on the leaves that they can be infected.

In conclusion, it might be stated that so far as the menace of citrus canker to the Citrus industry in the United States is concerned, with the exception of *Poncirus trifoliata*, none of the relatives of Citrus, native or introduced, discussed

above are sufficiently susceptible to warrant further attention. However, it is of scientific interest to know that *Pseudomonas citri* is not limited in its attack to the genus Citrus but can produce, under certain conditions, infections on a wide range of plants in the family Rutaceae to which Citrus belongs. In this connection, the plant breeder is furnished facts which may assist him in developing hybrids of a commercial nature which are resistant to citrus canker.

SUSCEPTIBILITY OF CITRUS FRUITS

The difference in the susceptibility of the two forms of *Citrus hystrix* DC has been consistent throughout the investigation. The round leaf form equals grapefruit in its susceptibility to canker, while the pointed leaf form shows some resistance to canker. Various forms of this obscure but large group should be tested further in the Philippine Islands where it is native, and undoubtedly, forms resistant to citrus canker will be found.

No changes were noted in the reaction of the citrons, lemons, and limes tested in the greenhouse. None of these plants were used in the field, so that their susceptibility to canker must be based on greenhouse results. While plants of the Ichang lemon were tested both in the greenhouse and field, no changes have occurred to modify previous reports.

All grapefruit plants tested are susceptible to citrus canker. Of the pummelos, the Hirado Buntan and Siamese have shown some resistance in that infection was almost wholly confined to the leaves with only an occasional spot on a young, growing twig.

Of the sweet orange group, no variety or species stands out as resistant, although they are not as susceptible as some of the other citrus fruits.

All of the plants belonging to the *Citrus nobilis* group show resistance to canker. In the majority of instances, canker was limited to scattering spots on the young leaves and on an occasional twig. The Cleopatra tangerine, which appears to be an excellent stock for some types of Citrus might after further trials be found suitable to replace the trifoliate orange as a stock.

As has been pointed out in a previous report, Tanaka [4] and Scott [5] have shown that there are several distinct strains of Satsuma grown in southern Alabama. The two most commonly found are the Owari and Ikeda. Scott describes them as follows:

> The fruits of the Owari are flat, thin skinned, depressed at both the blossom and stem ends, and practically seedless, maturing in Alabama the latter part of October or first of November. The leaves are very broad, particularly at the base, and the tree has an upright habit of growth.
>
> The fruits of the Ikeda variety are not as flat as those of the Owari, are not depressed at either the stem or blossom end, have a very coarse texture and a thick skin, and generally contain a few seeds. The season of the Ikeda is three or four weeks later than that of the Owari variety. The leaves are narrow, and the tree has a very spreading habit of growth.

From various sources reports of a difference in susceptibility between these strains were received, and in order to determine if they varied in susceptibility the following experiment was conducted:

During the early spring of 1918, thirty 3-year-old trees budded on *Poncirus trifoliata* of the Owari strain and a like number of the Ikeda strain were planted in the isolation field. As a control, thirty 3-year-old Duncan grapefruits budded on *Poncirus trifoliata* were planted between the two rows of Satsumas. Observations were made as to the number of trees infected and the parts of the plants attacked at intervals during the growing season over a period of three years. During this time the number of trees did not remain the same in that a number died or were cut out to make room for the remainder to grow. At the conclusion of the test, only 13 of each kind of tree remained. Since these trees were always surrounded by susceptible trees, natural infection was depended on entirely.

[4] TANAKA, T. VARIETIES OF THE SATSUMA ORANGE GROUP IN JAPAN. U. S. Dept. Agr. Bur. Plant Indus. Crop Physiol. and Breeding Inves. C. P. & B. I. Circ. 5, 10 p., illus. 1918.

[5] SCOTT, L. B. VARIETIES OF THE SATSUMA ORANGE GROUP IN THE UNITED STATES. U. S. Dept. Agr. Bur. Plant Indus. Hort. and Pomol. Inves. H. & P. Circ. 1, 7 p., 1918

At the conclusion of the investigation, no distinct difference in the susceptibility of the two strains of Satsumas was apparent. While the number of Ikeda trees infected earlier in the season was greater than the Owari, the reverse occurred later in the season, so that more of the Owari trees were infected than Ikeda. Further, infection was somewhat lighter on Owari than Ikeda although the percentage of Owari trees infected by canker was larger than that of Ikeda. Thus, from the results of this experiment conducted with a fair number of plants over a period of three years, absolutely no differences in the susceptibility of these two strains was apparent. With only a few exceptions, canker was limited to the young growing leaves and an occasional twig. On the other hand, the grapefruit plants were very badly infected at all times during the season, canker being found on the leaves, thorns, and round wood.

Citrus mitis Blanco proved to be more resistant than the Satsuma under field conditions. Canker at all times was limited to a few young leaves and an occasional twig. In the greenhouse experiments, the plants were more susceptible as twig infection was common and occasionally the round wood was attacked.

Likewise, the Kansu orange proved to be decidedly resistant. Very few canker spots on the leaves and no twig infection were noted. Of the other Citrus fruits tested, all proved to be susceptible.

In the continued search among the Citrus fruits for promising canker resistant types, the search will have to be confined to a few groups which show some promise of being resistant to canker.

RELATIVE SUSCEPTIBILITY OF THE CITRUS HYBRIDS

Both faustrime and faustrimon are more susceptible than *Microcitrus australasica* (F. Muell.) Swingle. Faustrimedin is not as susceptible in that infection is limited to a few spots on the leaves and an occasional twig.

All of the *Poncirus trifoliata* crosses, including citranges, citrumelo, citradia, citrandarin, citrunshu, cicitrange, and citraldin, are susceptible, and in no instance can any one number be selected as showing enough resistance to citrus canker to warrant its use as a canker resistant stock or orchard tree. While none of these hybrids are resistant to canker, several of them stand out above the common level and may be of some use for further breeding and crossing purposes. Throughout this investigation the Willets citrange has consistently shown less infection than any of the other citranges. The fact that it is one of the parents of the citrangequat, the most resistant hybrid so far found, substantiates to some extent these results.

On the whole, it can be stated that when *Poncirus trifoliata* is crossed with any of the more resistant Citrus species the resistance of the progeny is lessened.

Of the four citrange crosses, citrangequat, citranguma, citrangarin, and citrangedin, it happens that the second parent in each case is resistant to canker. Unfortunately, no hybrids were tested with a susceptible parent, so that we do not know what is the direct influence of the citrange parent on canker susceptibility. The citrangequat has consistently proved very resistant, in fact, as much so as its second parent. It appears to be the most promising canker resistant hybrid so far found. One disadvantage in the use of citrangequat as a stock has been the small number of seeds produced. However, Swingle and Robinson[6] have recently developed a method of propagation by the use of root cuttings, so that stock of this hybrid can now be rapidly developed. If it is found that it will make as desirable a stock as *Poncirus trifoliata*, it can replace the trifoliate orange, which at the present time is a menace to the final eradication of citrus canker in the Gulf Coast States. The other three citrange crosses, while they do not show as much

[6] SWINGLE, W. T., and ROBINSON, T. R. TWO IMPORTANT NEW TYPES OF CITROUS HYBRIDS FOR THE HOME GARDEN—CITRANGEQUATS AND LIMEQUATS. Jour. Agr. Research, 23: 229–238, illus. 1923.

resistance as the citrangequat, are sufficiently resistant to warrant further trials both as a stock and as orchard trees.

Limelo, orangelo, satsumelo, clemelo, siamelo, and tangelo, hybrids with grapefruit as one parent, vary in their susceptibility to canker. In no instance are any of these hybrids as resistant as Satsuma to citrus canker, although a few numbers are only slightly more susceptible. Because of the fact that one of these hybrids, the tangelo, is being grown commercially and has merits which are demanding attention from the orchardists and the public, the reaction of these hybrids to canker is of importance. Until further information is at hand it is safe to state that the tangelo and satsumelo, with some numbers of siamelo, show enough canker resistance to place them beside the mandarin oranges in their resistance to canker.

Likewise, the limequat and orangequat can be regarded as canker resistant as Satsuma. The orangequat has been observed very closely in the field because of its apparent similarity to the Satsuma, both in the type of growth and in its reaction to citrus canker and scab. Its relation to the Satsuma, if there is any, will not be known until fruit of the orangequat is produced and compared with Satsuma. Oranguma and siamar are slightly less resistant than the above hybrids.

Judging from the results obtained with the various hybrids of *Citrus mitis*, it appears while somewhat resistant itself, it does not carry this resistance in the hybrid. Thus, in the order of their susceptibility, we have sopomaldin, citraldin, calarin, and calashu, which are equally as susceptible as the other parent, grapefruit, trifoliate orange, tangerine, and Satsuma, respectively.

In the search for promising canker-resistant plants, the results of over four years' investigations seems to point to the fact that our best plants will come from the hybrids. Already one or two of the hybrids showing almost as much resistance as Satsuma have been set out in a small way. No doubt, as the result of further tests by the Office of Crop Physiology and Breeding Investigations, Bureau of Plant Industry, United States Department of Agriculture, other canker-resistant hybrids will be found.

DISCUSSION

In Tables I, II, and III we have listed each number tested, giving the type and number of plants used, its index of susceptibility, and the part or parts of the plant attacked. The index of susceptibility is the maximum that the plant has shown while it was under investigation. The same is true of the parts of plants attacked. Thus, the list as it stands records the maximum susceptibility shown by the plants of each individual number.

It must also be remembered that for the most part these plants consisted of small seedlings of budded plants. In only a few cases were the plants large enough to bloom and set fruit. Further, they were fertilized to stimulate new growth, for it was only on the new growth that canker developed in the majority of cases. Also during the greater part of the work many of the more susceptible plants were badly infected with canker, so that an epidemic condition existed both in the greenhouse and field, and consequently natural infections could be counted on at all times. Overwintering of canker in the field occurred each season, so that during the last three seasons very few of the plants were inoculated.

In all of our publications on citrus canker one point has been continually stressed, namely, that the plants must be in good growing condition for canker to infect. In other words, the plant must be making a good, vigorous growth to produce maximum infection. Therefore every means was used to keep the plants in a good, vigorous growing condition during the investigation.

Under grove conditions with older trees no doubt many of the numbers tested would show greater resistance to canker. However, the fact that under the conditions existing maximum susceptibility was obtained renders the results more valuable in that it gives us a truer index of susceptibility.

TABLE I.—*Relative susceptibility of the wild relatives of Citrus to canker*

Genus and species	C. P. B. No.[a]	Type of plant	Location	Number of plants	Results
Rutaceous plants not closely related to the genus Citrus					
Melia azadarach	Alabama	Seedling	Field	6	Negative.
Xanthoxylum bungei	11180	do	Greenhouse	1	Do.
Xanthoxylum sp	11269	do	do	1	Do.
Casimiroa edulis	7923	do	do	3	Leaves only at wounds.
		do	Field	1	Do.
Toddalia asiatica	11145	do	Greenhouse	1	Negative.
Glycosmis pentaphylla	2905	do	do	2	Do.
		do	Field	2	Do.
Clausena lansium	7936	do	Greenhouse	2	Leaves only at wounds.
		do	Field	4	Negative.
Chalcas exotica	7975A	do	Greenhouse	2	Leaves only at wounds.
	7980	do	Field	7	Negative.
Tribe Citreae, subtribe Aeglinae					
Aegle marmelos	7759	do	do	2	Do.
	7983	do	Greenhouse	2	Leaves only at wounds.
Aeglopsis chevalieri	7633	do	do	1	Negative.
		do	Field	1	Do.
	7772	Cutting	Greenhouse	2	Do.
Chaetospermum glutinosum	7138	Seedling	Field	2	Susceptible minus.
	7790	do	Greenhouse		Do.
		do	Field		Negative.
	7814	do	Greenhouse		Susceptible minus.
		do	Field		Negative.
Balsamocitrus dawei	2920	Budded[b]	Greenhouse	1	Do.
		do	Field	1	Do.
Subtribe Feroninae	7725	Budded[c]	do	1	Do.
Feronia limonia	2763	Seedling	Greenhouse	3	Leaves only, mostly at wounds.
		do	Field	5	Negative.
	11287	do	do	2	Do.
Feroniella lucida	7882	do	Greenhouse	2	Leaves only, mostly at wounds.
		do	Field	7	Do.
Subtribe Lavanginae					
Hesperethusa crenulata	2758	do	do	4	Susceptible minus.
	2759	do	Greenhouse	2	Do.
		do	Field	4	Do.
Triphasia trifolia	2689A	do	Greenhouse	2	Negative.
		do	Field	2	Do.
	7780	do	Greenhouse	1	Do.
Paramignya monophylla	7874	do	do	1	Leaves easily infected.
Severinia buxifolia	2760	Cutting	do	2	Negative.
		do	Field	17	Do.
Subtribe Citrinae	11822	Seedling	do	12	Do.
Citropsis schweinfurthii	7999	do	do	2	Negative.
	11260	do	Greenhouse	2	Leaves only at wounds.
		do	Field	5	Negative.
Atalantia citrioides	7534	Cutting	Greenhouse	4	Leaves easily infected.
		do	Field	4	Do.
Atalantia ceylonica	11225	Seedling	Greenhouse	1	Leaves and stems easily infected.
Poncirus trifoliata	Alabama	do	do	4	Extremely susceptible.
		do	Field	24	Do.
Eremocitrus glauca	7239	do	Greenhouse	4	Susceptible minus.
		do	Field	9	Do.
Fortunella margarita	7597	do	Greenhouse	3	Leaves only, resistant.
	11302	do	Field	3	Do.
	11327	do	do	2	Negative.
Fortunella japonica	11301	do	Greenhouse	3	Leaves only, resistant.
		do	Field	2	Do.
Fortunella crassifolia	7946D	do	do	3	Negative.
	11047	do	Greenhouse	6	Leaves only, resistant.
	11047A	do	Field	3	Negative.
Fortunella hindsii	11046A	do	Greenhouse	4	Susceptible minus.
		do	Field	8	Do.
	11319	do	do	6	Do.
Microcitrus australasica	7600	Cutting	Greenhouse	3	Do.
		Seedling	Field	5	Do.
	7600B	do	Greenhouse	2	Do.
		do	Field	5	Do.
Microcitrus australasica sanguinea.	7775B	do	Greenhouse	1	Do.
Microcitrus garrowayi	11008	Cutting	do	3	Do.
		do	Field	2	Do.
Microcitrus australis	7307	do	Greenhouse	2	Do.
		do	Field	3	Do.
	7427	Seedling	Greenhouse	1	Do.
		do	Field	5	Do.
	7775E	Cutting	do	2	Do.

[a] Crop Physiology and Breeding Investigations. [b] On *Aeglopsis chevalieri*. [c] On *Aegle marmelos*.

TABLE II.—*Relative susceptibility of citrus fruits to canker*

TABLE II.—*Relative susceptibility of citrus fruits to canker*

Genus and species	C. P. B. No.[a]	Type of plants	Location	Number of plants	Results	Remarks
C. hystrix, pointed-leaf form	7727	Seedling	Field	2	Susceptible minus	Leaves and occasional twig.
	7872	do	Greenhouse	2	do	Do.
		do	Field	12	do	Do.
C. hystrix, round-leaf form	2881	do	Greenhouse	1	Susceptible plus	Leaves, twigs, and round wood.
	7831	do	do	2	do	Do.
		do	Field	2	do	Do.
C. medica, Citron of Commerce	7768	Cutting	Greenhouse	2	Susceptible	Do.
	7836	Seedling	do	1	do	Do.
"Sidro"	7816	do	do	2	do	Do.
"Odorata"	11294	do	do	2	do	Do.
"Nana"	11281	do	do	2	do	Do.
"Etrog"	11178	do	do	2	do	Do.
Citrus sp. small lemon	7833	do	do	2	do	Do.
Sweet	1158	do	do	2	do	Do.
"Davao"	7837	do	do	2	do	Do.
"Limon real" No. 18	7819	do	do	2	do	Do.
Ichang	11204-A	do	do	3	do	Leaves and occasional twig.
		do	Field	4	do	Leaves, twigs, and occasionally round wood.
C. limonia, large lemon	11291	do	Greenhouse	4	do	Leaves and occasional twig.
C. aurantiifolia, sour lime	7832	do	do	2	do	Leaves, twigs, and round wood.
C. grandis, grapefruit	7338	do	do	2	do	Leaves and occasional twig.
	7903	do	Field	6	Susceptible plus	Leaves, twigs, and round wood.
	11170	do	Greenhouse	2	do	Do.
Sullivan	Alabama	do	do	5	do	Do.
	11001	do	Field	3	do	Do.
	11054	do	do	3	do	Do.
Shaddock Florida	11255	do	do	3	do	Leaves, twigs, and round wood.
Pummelo "pomelo"	7834	do	Greenhouse	2	do	Do.
Hirado Buntan	7903	do	do	3	Susceptible minus	Leaves and occasional twig.
		do	Field	3	do	Do.
Indian	2968A	do	Greenhouse	2	Susceptible plus	Leaves, twigs, and round wood.
		do	Field	2	do	Do.
	11163	do	do	3	do	Do.
	11168	do	do	2	do	Do.
Siamese	6111	Budded[b]	Greenhouse	1	Susceptible minus	Leaves only.
		do	Field	1	Susceptible	Do.
	11201	Seedling	Greenhouse	2	do	Do.
		do	Field	3	Susceptible minus	Leaves and occasional twig.
Chinese Orangedale	11212U	do	do	3	Susceptible plus	Leaves, twigs, and round wood.
French Martin	11213S	do	do	1	do	Do.
Mark's	11061	do	Greenhouse	2	do	Do.
		do	Field	3	Susceptible	Leaves, twigs, and occasionally round wood.
	11217F	do	do	3	do	Do.
	11217G	do	do	3	do	Do.

[a] Crop Physiology and Breeding Investigations.

[b] On *Citropsis schweinfurthii*.

TABLE II.—*Relative susceptibility of citrus fruits to canker*—Continued

Genus and species	C. P. B. No.	Type of plants	Location	Number of plants	Results	Remarks
C. sinensis, orange	66A	Seedling	Greenhouse	2	Susceptible	Leaves and occasional twig.
Grenadine	7773	do	do	3	do	Do.
Parsons Brown	11324	do	do	2	do	Do.
Navel	45344	Budded[4]	Field	1	do	Do.
Temple	11164	Seedling	do	2	do	Do.
Japanese No. 1	11198	do	do	3	do	Leaves, twigs, and occasionally round wood.
Japanese No. 2	11199	do	do	3	do	Do.
Sekkan	11023	do	do	3	do	Leaves and occasional twig.
Pankan	7560A	do	do	3	do	Do.
Tankan	7562	do	do	6	do	Do.
South Carolina	11028	do	do	2	Susceptible plus	Leaves, twigs, and round wood.
Kirkland	11313	do	do	6	Susceptible	Leaves and occasional twig.
C. nobilis, King of Siam	2105	do	Greenhouse	2	Susceptible minus	Do.
	11384	do	Field	6	do	Do.
Naranjita	7830	do	Greenhouse	2	do	Do.
C. nobilis, unshiu, Satsuma	Alabama	Budded[4]	do	2	do	Leaves only.
C. nobilis, deliciosa, Tangerine	11195	Seedling	do	3	do	Do.
Cleopatra	11338	do	do	2	do	Do.
		do	Field	18	do	Leaves and occasional twig.
C. mitis, Calamondin	7065	do	Greenho	3	do	Do.
	11265	do	do	2	do	Do.
		do	Field	4	do	Leaves only.
	11312	do	do	17	do	Do
	44305	do	Greenhouse	2	do	Leaves and occasional twig.
Citrus sp. Kansu	7908	do	Field	3	do	Leaves only.
		do	do	1	do	Do.
	11242	do	Greenhouse	3	do	Leaves and occasional twig.
	11298	do	Field	3	do	Leaves only.
		Budded[4]	do	1	do	Do.
		Budded[4]	do	1	do	Do.
Citrus sp. Natsu-mikan	11184	Seedling	Greenhouse	3	Susceptible	Leaves, twigs, and occasionally round wood.
		do	Field	3	do	Do.
	11197	do	do	4	do	Do.
	11337	do	do	3	do	Do.
Citrus sp. Talamisan No. 11	7827	do	Greenhouse	2	Susceptible plus	Leaves, twigs, and round wood.
Colo-colo	7820	do	do	2	do	Do.
Tegi-tegi	7818	do	do	1	Susceptible minus	Leaves and occasional twig.
Naranja	7829	do	do	3	Susceptible	Do.
		do	Field	2	do	Leaves, twigs, and occasionally round wood.
Narute	11196	do	do	3	do	Do.
C. excelsa	11280	do	Greenhouse	2	do	Do.

[2] On *Poncirus trifoliata*. [4] On *Citrus grandis*. [5] On *Severinia buxifolia*.

TABLE III.—*Relative susceptibility of citrus hybrids to canker*

TABLE III.—*Relative susceptibility of citrus hybrids to canker*

Hybrid	C. P. B.[a] No.	Type of plant	Location	Number of plants	Results	Remarks
Faustrime (M. australasica×C. aurantifolia)	49819	Cutting	Greenhouse	1	Susceptible	Leaves, twigs, and round wood.
	49823	do	do	1	do	Do.
Faustrimon (M. australasica×C. limonia)	49835	do	do	1	do	Do.
	49841	do	do	1	do	Do.
	49843	do	do	1	do	Do.
	49844	do	do	1	do	Do.
Faustrimedin (M. australasica×C. mitis)	47431	do	do	2	do	Leaves and occasional twig.
		do	Field	2	do	Do.
Citrange Colman (P. trifoliata×C. sinensis)	772A C	Seedling	do	2	Susceptible plus	Leaves, twigs, and round wood.
	7896	do	Greenhouse	2	do	Do.
Cunningham	1418A A	do	Field	2	do	Do.
	7965	do	Greenhouse	2	do	Do.
Morton	761A C	do	do	2	do	Do.
	771A	do	do	2	do	Do.
Rusk	716	do	Field	2	do	Do.
	7895	do	do	10	do	Do.
	7895Y	do	Greenhouse	2	do	Do.
	7956	do	do	2	do	Do.
		do	Field	5	do	Do.
	11030	do	Greenhouse	3	do	Do.
	44980	do	do	2	do	Do.
		do	Field	1	do	Do.
Willits	777A B	do	do	3	Susceptible	Leaves and occasional twig.
	7897	do	Greenhouse	2	do	Do.
	7960	do	do	2	do	Do.
Savage	779A A	do	Field	3	Susceptible plus	Leaves, twigs, and round wood.
	7898	do	do	4	do	Do.
	7961	do	Greenhouse	2	do	Do.
		do	Field	5	do	Do.
Sanford	1423A B	do	do	3	do	Do.
	7963	do	Greenhouse	2	do	Do.
Rustic	7934A	do	do	2	do	Do.
Etonia	749A B	do	Field	2	do	Do.
Citrange	1416	do	Greenhouse	2	do	Do.
	1425A B	do	Field	1	do	Do.
	43434	do	Greenhouse	2	do	Do.
	43480	do	do	2	do	Do.
	43931	do	do	2	do	Do.
Citrumelo (P. trifoliata×C. grandis)	1425A A	do	Field	2	do	Do.
	4475A	do	Greenhouse	1	do	Do.
	4493	do	do	2	do	Do.
	4554	do	do	4	do	Do.
	4564	do	do	1	do	Do.
Citradia (P. trifoliata×C. aurantium)	50850	do	do	2	Susceptible	Leaves, twigs, and occasionally round wood.

[a] Crop Physiology and Breeding Investigations.

[b] On *Poncirus trifoliata*.

[c] On *Citrus grandis*.

[d] On *Severinia buxifolia*.

TABLE III.—*Relative susceptibility of citrus hybrids to canker*—Continued

Hybrid	C. P. B. No.	Type of plant	Location	Number of plants	Results	Remarks
Citrandarin (P. trifoliata×C. nobilis)	40175A	Seedling	Field	4	Susceptible	Leaves, twigs, and occasionally round wood.
	40210	do	Greenhouse	3	do	Do.
	40303C	do	do	2	do	Do.
	40315	do	do	2	do	Do.
	40368B	do	do	2	do	Do.
	48529	Budded [b]	do	2	do	Do.
Citrandarin (P. trifoliata×C. nobilis unshiu)	49720	Seedling	Field	1	do	Do.
	49721	do	do	1	do	Do.
	49724	do	do	1	do	Do.
	49726	do	do	1	do	Do.
	49732	do	do	1	do	Do.
	49737	do	do	1	do	Do.
	49746	do	do	1	do	Do.
	49748	do	do	1	do	Do.
Citrandarin (P. trifoliata×C. nobilis deliciosa)	49624	do	do	1	do	Do.
	49625	do	do	1	do	Do.
	49644	do	do	1	do	Do.
	49663	do	do	1	do	Do.
	49664	do	do	1	do	Do.
	49686	do	do	1	do	Do.
	49688	do	do	1	do	Do.
	49695	do	do	1	do	Do.
	49699	do	do	1	do	Do.
	49712	do	do	1	do	Do.
Citrunshu (P. trifoliata×C. nobilis unshiu)	49607	do	do	1	do	Leaves, twigs, and round wood.
	49608	do	do	1	do	Do.
	49611	do	do	1	do	Do.
	49615	do	do	1	do	Do.
	49620	do	do	1	do	Do.
	49623	do	do	1	do	Do.
		do	do	1	do	Do.
	51102	Budded [b]	do	1	do	Do.
Cicitrange (P. trifoliata×Colman citrange)	48290	Seedling	Greenhouse	2	Susceptible plus	Do.
	48316	do	do	3	do	Do.
Citrangequat (Willits citrange×F. margarita)	48003	Budded [c]	Field	1	No infection	
	48005	Budded [d]	do	1	Very resistant	Leaves only.
	48007	Budded [e]	do	1	No infection	
	48010	Seedling	Greenhouse	2	Very resistant	Do.
	48010	Budded [c]	Field	1	No infection	Do.
	48010D2	Seedling	Greenhouse	3	Very resistant	Do.
Citrangequat (Willits citrange x F. margarita)	48010D5	Seedling	Field	2	do	Do.
	48010E1	do	do	4	do	Do.
	48010E	do	do	1	No infection	
	48010E	Budded [f]	do	1	do	
	48010F	Seedling	do	4	Leaves only	Very resistant.
	48011	Budded [d]	do	1	No infection	
	48379	do	do	1	Very resistant	Leaves only.

	48379	do	do	1	Very resistant	Leaves only.
	48888	do	do	1	do	Do.
	48897	do	do	1	do	Do.
Citranguma (Morton citrange×C. nobilis unshiu)	48054	Budded [e]	do	1	Susceptible	Leaves, twigs, and occasionally round wood.
	48055	Seedling	Greenhouse	2	Resistant	Leaves and occasional twig.
	48055A	do	do	1	Susceptible minus	Leaves, twigs, and occasionally round wood.
	48217	Budded [b]	Field	1	Susceptible	Do.
	48218	Budded [e]	do	1	do	Do.
	49773	Seedling	do	1	do	Do.
		Budded [h]	do	1	do	Do.
		Budded [i]	do	1	do	Do.
Citrangarin (Sanford citrange×C. nobilis deliciosa)	49776	Seedling	do	1	Susceptible minus	Leaves and occasional twig.
		Budded [h]	do	2	do	Do.
Citragedin (Citrange×C. mitis)	48045	Seedling	Greenhouse	1	do	Leaves only.
		Budded [b]	Field	1	do	Do.
	50207	do	do	1	do	Leaves and occasional twig.
	50479	do	do	1	do	Do.
	50485	do	do	1	do	Do.
		Seedling	do	1	do	Leaves only.
	50486	Budded [b]	do	1	do	Do.
		do	do	1	do	Leaves and occasional twig.
	50493	do	do	1	do	Do.
	50495	do	do	1	do	Do.
		Budded [d]	do	1	do	Leaves only.
	50498	Budded [b]	do	1	do	Do.
	50500	do	do	1	do	Leaves and occasional twig.
		Budded [j]	do	1	do	Leaves only.
	50501	do	do	1	do	Leaves and occasional twig.
		Budded [b]	do	1	do	Do.
		Budded [d]	do	1	do	Do.
Limequat (C. aurantifolia×F. margarita)	48787A	Seedling	Greenhouse	2	do	Do.
	48787B	do	do	3	do	Do.
	48792E	do	do	3	do	Do.
		do	Field	2	do	Do.
	48798	Budded [b]	do	1	do	Do.
Limelo (C. aurantifolia×C. grandis)	40502	Seedling	Greenhouse	2	Susceptible plus	Leaves, twigs, and round wood.
	40526A	do	do	2	do	Do.
	40567B	do	do	2	do	Do.
Bigaraldin (C. aurantium×C. mitis)	50352	Budded [b]	Field	1	Susceptible	Leaves, and occasional twig.
	50355	Budded [d]	do	1	do	Do.
Orangelo (C. sinensis×C. grandis)	1068	Seedling	do	3	do	Leaves, twigs, and occasionally round wood.
	49107	Budded [d]	do	1	Susceptible plus	Do.
	49132	do	do	1	do	Do.
Oranguma (C. sinensis×C. nobilis unshiu)	52117	do	do	1	Susceptible	Leaves and twigs.
	52118	Budded [i]	do	1	do	Do.
	52129	Budded [i]	do	1	do	Do.
	52131	Budded [d]	do	1	do	Do.
	52142	Budded [b]	do	1	do	Do.
	57121	Budded [i]	do	1	do	Do.

[b] On *Poncirus trifoliata*.
[e] On *Severinia buxifolia*.
[d] On *Citrus grandis*.
[e] On *Citrus aurantifolia*.
[f] On *Citropsis schweinfurthii*.
[g] On *Citrus limonia*.
[h] On clemelo.
[i] On Savage citrange.
[j] On Rusk citrange.
[k] On *Citrus nobilis deliciosa*.

TABLE III.—*Relative susceptibility of citrus hybrids to canker*—Continued

Hybrid	C. P. B. No.	Type of plant	Location	Number of plants	Results	Remarks
Orangequat (C. sinensis×F. margarita)	49956	Budded [1]	Field	1	Susceptible	Leaves only.
Satsumelo (C. nobilis unshiu×C. grandis)	50312	Budded [b]	do	1	do	Leaves and occasional twig.
	48812	Budded [c]	do	1	do	Do.
	50304	Budded [d]	Greenhouse	1	do	Leaves only.
		Budded [e]	Field	1	do	Leaves and occasional twig.
	52001R1	do	do	1	do	Do.
	5200312	do	do	1	do	Do.
	52009A2	Budded [d]	Greenhouse	1	do	Leaves only.
	52009G5	do	do	1	do	Leaves, twigs, and round wood.
	52009Q1	Budded [e]	Field	1	do	Leaves and occasional twig.
	52009V4	do	do	1	do	Do.
	52010F1	do	do	1	do	Do.
	52011M1	do	do	1	do	Do.
	52011R1	Budded [d]	do	1	do	Leaves only.
	52011Y4	do	do	1	do	Leaves, twigs, and round wood.
	52022P1	Budded [e]	do	1	do	Leaves and occasional twig.
	52032H1	do	do	1	do	Do.
	52304	Budded [b]	do	1	do	Do.
Clemelo (C. nobilis deliciosa×C. grandis)	49006	Seedling	Greenhouse	1	Susceptible plus	Leaves, twigs, and round wood.
	49013	Budded [b]	do	1	do	Do.
		Budded [d]	do	1	do	Leaves and twigs.
	49025	Budded [b]	do	1	do	Do.
		Budded [d]	do	1	do	Do.
	49028	Budded [b]	do	1	do	Do.
	49032	do	do	1	do	Do.
	49038	do	do	1	do	Leaves, twigs, and round wood.
	49043	Budded [d]	do	1	do	Leaves and twigs.
	49049	Budded [b]	do	1	do	Leaves, twigs, and round wood.
Siamelo (C. nobilis×C. grandis)	47355B	Seedling	Greenhouse	2	Susceptible	Leaves and twigs.
	50520	Budded [d]	Field	1	do	Leaves, twigs, and round wood.
	50535	do	do	1	Susceptible plus	Do.
		do	do	1	Susceptible	Leaves and twigs.
	50551	do	do	1	do	Do.
	51531	do	do	1	do	Do.
	51565	do	do	1	do	Do.
	51584	do	do	1	do	Do.
	51586	do	do	1	do	Do.
	51588	Seedling	Greenhouse	1	do	Do.
	51598	do	do	1	do	Do.
	51947		Field	1	do	Do.
	51969	Budded [d]	do	1	do	Do.
	52007–O6	do	do	1	do	Do.
	52007–P3	do	do	1	do	Do.
	52007–P11	do	do	1	do	Do.

	51909	Budded [c]	do	1	do	Do.
	52007–O6	do	do	1	do	Do.
	52007–P3	do	do	1	do	Do.
	52007–P11	do	do	1	do	Do.

	52013–S9	do	do	1	Susceptible plus	Leaves, twigs, and round wood.
	52021–A5	do	do	1	Susceptible	Leaves and twigs.
Siamor (C. nobilis×C. sinensis)	52020–C1	do	do	1	do	Leaves and occasional twig.
	52029–C11	do	do	1	do	Do.
	52029E	do	Greenhouse	1	do	Do.
Calashu (C. mitis×C. nobilis unshiu)	50309	Budded [b]	Field	1	Susceptible minus	Leaves only.
		Budded [f]	do	1	do	Do.
		Budded [d]	do	1	do	Do.
Citraldin (P. trifoliata×C. mitis)	50130	do	do	1	Susceptible	Leaves and twigs.
Sopomaldin (C. grandis×C. mitis)	50378	do	do	1	Susceptible plus	Leaves, twigs, and round wood.
	50393	do	do	1	Susceptible	
		Budded [i]	do	1	Susceptible plus	Do.
Calarin (C. mitis×C. nobilis deliciosa)	50314	Budded [b]	do	1	Susceptible	Leaves and twigs.
Tangelo, Thornton (C. nobilis deliciosa×C. grandis)	L715	Seedling	Greenhouse	2	do	Leaves, twigs, and occasionally round wood.
	714B	do	Field	3	do	Do.
	11034	do	Greenhouse	2	do	Do.
Sampson	L789	do	do	2	do	Do.
	7764	do	Field	5	do	Do.
	11037	do	do	1	do	Do.
Vero	11340	do	do	10	do	Do.
Tangelo	L16	do	Greenhouse	3	do	Do.
	L461	do	do	1	do	Do.
	1191	do	do	1	do	Do.
	1230	do	do	1	do	Do.
	1257A	do	do	2	do	Do.
	1262B	do	do	1	do	Do.
	1348A	do	do	1	do	Do.
	7161	do	do	1	do	Do.
	7675C	do	do	1	do	Do.
	40971A	do	do	1	do	Do.
	52018C2	Budded [d]	do	1	do	Do.
	52018F2	do	do	1	do	Do.
	49425	do	Field	1	do	Do.
	49818I14	do	do	1	do	Do.
	49879	do	do	1	do	Do.
	50312	Budded [b]	do	1	do	Do.
	52016C12	Budded [d]	do	1	do	Do.
	52016D6		do	1	do	Do.
	52016E4	do	do	1	do	Do.
	52018A4	do	do	1	do	Do.
	52018N1	Budded [m]	do	1	do	Do.
	5201883	Budded [m]	do	1	do	Do.
	52018U2	do	do	1	do	Do.
	52018X17	do	do	1	do	Do.
False hybrids	11 nos.	Seedling	Greenhouse	20	Susceptible plus	Leaves, twigs, and round wood.
	10 nos.	do	Field	14	do	Do.

[b] On *Poncirus trifoliata*.
[c] On *Citrus limonia*.
[d] On *Citrus grandis*.
[f] On Savage citrange.
[i] On Rusk citrange.
[m] On Natsu-mikan.

RELATION OF ENVIRONMENTAL FACTORS TO CITRUS SCAB CAUSED BY CLADOSPORIUM CITRI MASSEE[1]

By GEORGE L. PELTIER, *formerly Plant Pathologist, Alabama Agricultural Experiment Station, and Agent, Bureau of Plant Industry, United States Department of Agriculture*, and WILLIAM J. FREDERICH, *formerly Assistant Pathologist, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

In connection with citrus canker investigations conducted by the writers, an opportunity was afforded to observe the behavior of citrus scab caused by *Cladosporium citri* Massee under the weather conditions prevailing in southern Alabama for four seasons. In the following pages an attempt is made to correlate field observations with studies made under controlled conditions in the laboratory and greenhouse on the pathogene, on the host plant, and on infection and development of the disease.

HISTORY AND DISTRIBUTION

Scab attacks the young fruit, leaves, and occasionally the shoots of Citrus plants. The effect of scab on the fruits is the most important aspect of the disease from an economic viewpoint, in that scabby fruit either results in lower market value or in cull fruits.

What probably constitutes the earliest record of scab was found by Lee (*5*)[2] on a herbarium specimen of *Citrus nobilis* Lour. collected at Nagasaki, Japan, in 1863. He states that the early occurrence of this disease in Japan and the general distribution of citrus scab throughout the Citrus-growing regions of South China indicate that this disease may be indigenous to the Orient.

Reinking (*7*, *8*), in a plant-disease survey of southern China found scab generally prevalent in this region, which substantiates Lee's conclusions. Considerable work has been done on scab in Japan, as it is there one of the more important diseases of Citrus, but owing to the inaccessibility of the Japanese literature it is difficult to obtain much information regarding the history of scab in that country. No doubt, as in the case of the citrus canker, Japan has indirectly served as the center for the distribution of scab to some of the countries in which the disease is now present. For several decades large exportation of Citrus nursery stock to various parts of the world has been made from several Japanese ports. Thus it is possible that Japan served as the center for scab distribution to Australia, the Gulf Coast section of the United States, and South Africa, as it did in the case of canker. So far as is known, no scab has ever been reported from California and the Philippine Islands, although there is every reason

[1] Received for publication February 16, 1924. Published with the approval of the Director of the Alabama Agricultural Experiment Station. The paper is based on cooperative investigations between the Office of Crop Physiology and Breeding Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Plant Pathology, Alabama Agricultural Experiment Station. Presented before the thirteenth annual meeting of the American Phytopathological Society, Toronto, Canada, December 28 to 31, 1921, and abstracted in Phytopathology 12:57, 1922. Since that time two papers dealing with certain phases of the work discussed have appeared, to wit:

WINSTON, J. R.

1923. CITRUS SCAB: ITS CAUSE AND CONTROL. U. S. Dept. Agr. Bul. 1118, 38 p., illus.

TANAKA, T.

1923. A BRIEF HISTORY OF CITRUS SCAB IN JAPAN. Phytopathology 13:492-495.

[2] Reference is made by number (italic) to "Literature cited," p. 254.

to assume that scab has been introduced into these countries on nursery stock. Further, Reinking (*8*) does not mention the presence of scab in the Citrus-growing regions of Siam and Indo-China.

Fawcett (*2*) summarized the California situation as follows: "This disease (scab) has never been found in California. Before the strict quarantine laws were passed, thousands of sour orange trees with their leaves affected with scab were brought into California, but the new foliage came out free from attack. It would appear that this fungus is unable to persist in a climate like that of California."

According to Swingle and Webber (*9*), scab appeared in Florida about 1884 and spread rapidly over the State and into Louisiana. They state that it was probably introduced into America from Japan. Since that time it has been introduced from Florida and Louisiana, or direct from Japan on the many importations of nursery stock, especially Satsuma (*Citrus nobilis unshiu* (Makino) Swingle, into the Gulf Coast States of Alabama, Mississippi, and Texas. Thus, at the present time, scab is one of the more important diseases of Citrus fruits in the Gulf Coast States, although fortunately it can be controlled by spraying. Scab has also been reported from India, Formosa, the West Indies, Paraguay, the Canal Zone, Yucatan, and Hawaii.

Scab is a rather serious disease in the Gulf Coast States, South China, Japan, and parts of the West Indies. So far it has not been reported from the Citrus-growing regions of the Mediterranean countries, California, and the Philippine Islands. The absence of scab in these localities is an interesting phase of the scab situation. That scab has been introduced on nursery stock into California and the Philippine Islands, and possibly the Mediterranean countries without gaining a foothold, indicates that there are certain factors which prevent the successful propagation of citrus scab in these localities.

INFLUENCE OF ENVIRONMENTAL FACTORS UNDER CONTROLLED CONDITIONS

ON THE PATHOGENE

Fawcett (*1*, *2*) has continually emphasized the fact that *Cladosporium citri* differs from other species of Cladosporium, and especially *C. herbarum* Lk., which is usually associated with *C. citri* on old scab spots. In fact, *C. herbarum* has in some cases been mistaken for the causal organism. He has so well described the difference in appearance of these organisms both in pure culture and on the plants themselves, that it will be sufficient to state here that *C. citri* is an unusual Cladosporium, which makes an extremely small and slow growth in pure culture.

The optimum temperature, as worked out by Fawcett (*4*) for the best development of the fungus in pure culture, is 21° C. (69.8°F.). Growth occurs in culture within a range of temperatures between 13.5° and 32° C. (56° and 89.6° F.). Judging from Fawcett's results, spore production in pure cultures seems to be limited to temperatures between 16° and 27.5° C. (60.8° and 81.5° F.).

It has also been observed by Fawcett (*1*) that the spores of this fungus germinate readily both in tap water and on agar; germination of the spores beginning in from 5 to 24 hours. It is not known whether the spores will germinate in a saturated atmosphere or at lower humidities.

ON THE HOST PLANTS

The results obtained by the senior writer (*6*) at the University of Illinois indicate that each type of Citrus plant reacts differently under similar environmental conditions. Under controlled conditions, grapefruit plants (*Citrus grandis*

(L.) Osbeck) made an extremely slow growth at 18° C. (59° F.), while the other plants tested did not start until a temperature of 20° C. (68° F.), was reached. Even at this temperature *C. mitis* Blanco, a native of the Philippine Islands only made a slow growth. At 30° C. (86° F.) all plants tested developed rapidly. Above this point the growth of plants of the grapefruit type were inhibited, while *Poncirus trifoliata* (L.) Raf. made a good growth.

It was also found that at 15° C. (59° F.), grapefruit plants, although making an extremely slow growth matured their foliage rapidly, in most instances within a week's time, while at 30° C. (86° F.) growth was rapid, but the period of leaf maturation was extended over a period of about two weeks. The mature leaves at the low temperature were only from one-fourth to one-half the size of those on plants held at 30° C. (86° F.). The small leaves maturing at the lower temperature retained their same size when transferred to higher temperatures.

Plants held at 30° C. (86° F.) were inhibited in their growth when transferred to lower temperatures. The growth of citrus plants was inhibited when held at a high temperature during the day and at a low temperature during the night.

ON INFECTION AND DEVELOPMENT OF THE DISEASE

As far as the writers are aware, no one has successfully infected Citrus plants with *Cladosporium citri* under field conditions. Even in the greenhouse, infection has not always been successful. In practically all cases the only successful infections which have been produced under greenhouse conditions have been made on plants held under bell jars, or where abundant moisture on the surface of the leaves was supplied for a period of several days. In this connection it should also be stated that only the young leaves have been infected.

In the experiments reported by Fawcett (*4*), and in the observations made by the senior writer, the two factors of young growth and sufficient moisture for initial infection have always been supplied. Fawcett inoculated his plants and placed them in the temperature chambers, with sufficient moisture, for a period long enough for initial infection to take place. In the senior writer's observations on scab, the plants were held in temperature cases in a saturated atmosphere under bell jars.

Fawcett (*4*) has shown that the temperature limits for successful infection of sour orange and pummelo plants under controlled conditions lies between 16° and 23° C. (60.8° and 74.3° F.). No infections were obtained at 14° (57.2° F.) or below, nor at 24.5° C. (76.1° F.) or above, so that infection is limited to a rather narrow range of temperature. The largest number of scab spots occurred at the temperatures between 18.5° and 21° C. (65.3° and 69.8° F.).

In the senior writer's experiment with citrus canker (*6*) optimum conditions for scab development were afforded and typical scab spots did occur naturally on some of the plants growing in the temperature cases at 15° and 20° C. (59° and 68° F.). No scab appeared on plants held at temperatures of 10° C. (51° F.) or below, nor at 25° C. (77° F.) or above. At 15° C. (59° F.) scab spots occurred only on the grapefruit plants showing new growth. It should be noted that grapefruit were the only plants making any growth at this temperature. At 20° C. (68° F.) scab appeared on grapefruit, calamondin, and citrange plants. The spots were much more numerous on grapefruit than at 15° C. (59° F.). In all cases, scab was limited to the plants with young leaves.

The results show that when abundant moisture is supplied and young growth is present, infection of Citrus plants by *Cladosporium citri* is limited to temperatures between 15° C. and 23.5° C. (59° and 74.3° F.), a range of less than 10 degrees. The optimum for the best and greatest development of scab appeared to be about 20°–21° C. (68°–69.8° F.), which is also the optimum found by Fawcett (*4*) for the fungus in culture.

INFLUENCE OF ENVIRONMENTAL FACTORS UNDER FIELD CONDITIONS

ON THE HOST PLANTS

Citrus scab is usually prevalent during the development of the first spring growth. Under controlled conditions the maximum temperature for successful infection is about 75° F. Under Alabama conditions a mean weekly temperature of 75° F. and above is reached and the first spring growth of the plants is usually completed by the first of June. For the purpose of our discussion, then, we are interested primarily in the behavior of the plants during the interval required for the development of the first growth period.

It might be of interest in this connection to correlate the appearance of new growth of the more common Citrus plants under Alabama conditions, with the prevailing temperatures recorded at Mobile for the first five months of the years 1914 to 1920.

In Table I are listed the weekly mean temperatures from January 1 through June 10 for these years. In figure 1 the weekly mean temperatures for 1918 and 1916 are plotted with the two late seasons of 1915 and 1920 for comparison.

In 1915 no weekly mean temperatures suitable for active growth of Citrus plants were recorded until the week of April 9–15, when the mean rose to 67° F. and proceeded rapidly upward. This temperature was sufficient to force all plants into activity at practically the same time.

During the early part of 1916 the weekly mean temperature rose to 59° F. and above on three separate occasions, each of which was sufficient to force active growth of grapefruit plants but not Satsuma or trifoliate orange. In each case, however, these temperatures were followed by killing frosts. It was not until the week of March 19–25 that the weekly mean temperature was high enough to force new growth. The mean for this week was 68° F. which was high enough to force all Citrus plants into active growth.

While January, 1918, was conspicuous for its low temperatures, weekly mean temperatures for forcing and continuing growth of grapefruit plants occurred from the middle of February on. By the last of February the weekly mean temperatures were high enough for Satsuma and trifoliate orange to develop. Notes on the plants made in the isolation field on March 8 showed that the majority, which survived the low temperatures of January, were in full leaf or starting growth. In fact, scab was observed on a number of plants on this date. Without any question, the season of 1918 was the earliest under discussion.

During the 1920 season temperatures high enough to cause active growth of grapefruit plants and swelling of the trifoliate orange and Satsuma buds occurred during periods in January and February. In fact, grapefruit plants were almost in full leaf when killed back by the freezes of February 15 and 16. The lowest temperatures of the season occurred during the first week in March. All new growth which occurred prior to that time was killed back. With the advent of higher weekly mean temperatures it was some time before new growth started. The late season of 1920, then, was due to the destructive frosts the first week in March. The development of the uninjured buds was also delayed until suitable temperatures were again at hand. To summarize, most of the plants were in full leaf or were starting growth the week ending February 4 in 1918, March 25 in 1916, April 1 in 1920, and April 15 in 1915.

From the above notes it can be clearly seen that the dormancy of Citrus plants during the early part of the year is variable and depends to a large extent on the prevailing temperatures. The seasons of 1914, 1915, and 1920 can be

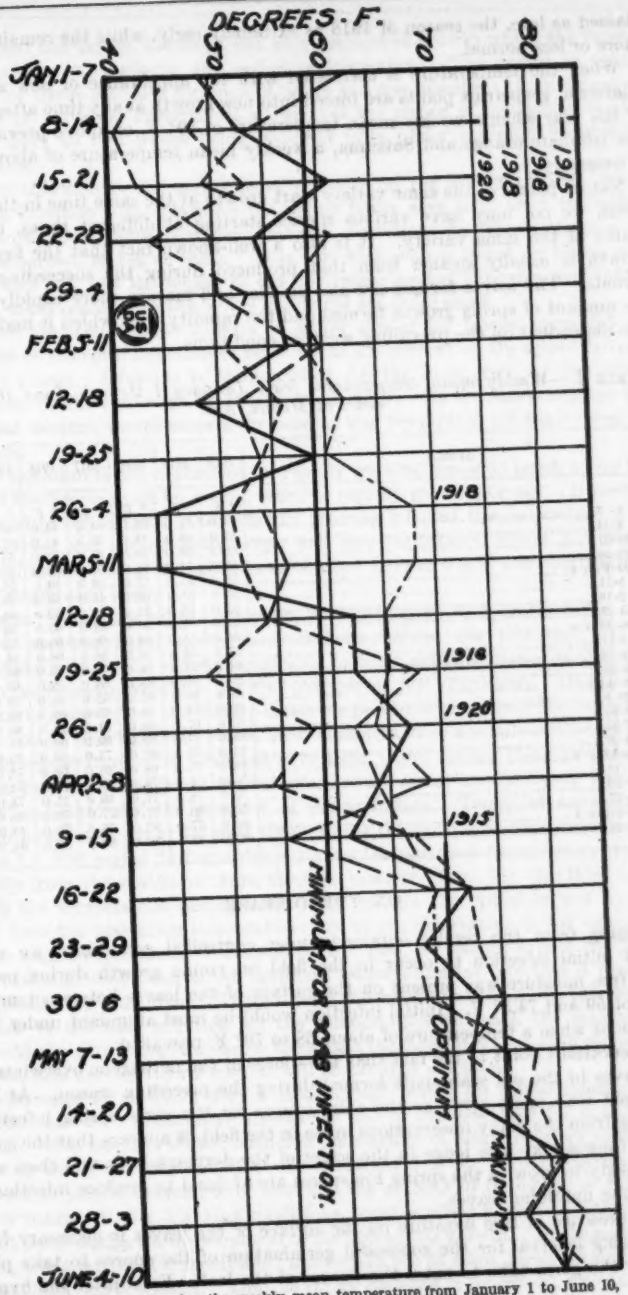


FIG. 1.—Graph showing the weekly mean temperature from January 1 to June 10, for the years 1915, 1916, 1918, and 1920 at Mobile, Ala., together with the minimum, optimum, and maximum temperatures for citrus-scab infection

classed as late, the season of 1918 as extremely early, while the remainder were more or less normal.

When the temperature is correlated with the appearance of new growth in Alabama, grapefruit plants are forced into new growth at any time after the first of the year when a weekly mean temperature of 59° F. or above prevails. For the trifoliate orange and Satsuma, a weekly mean temperature of above 65° F. is necessary.

Not all plants of the same variety start growth at the same time in the spring. Thus, we not only have various species starting at different times, but even plants of the same variety. It is also a well-known fact that the first spring growth is usually smaller than that produced during the succeeding growth periods. The leaves formed are also smaller and mature more rapidly. Thus the amount of spring growth formed and the rapidity with which it matures are also dependent on the prevailing weather conditions.

TABLE I.—*Weekly mean temperatures from January 1 through June 10, for 7 years at Mobile, Ala.*

Month	1914	1915	1916	1917	1918	1919	1920
	° F.	° F.	° F.	° F.	° F.	° F.	° F.
Jan. 1–7	44.0	51.0	63.0	61.0	44.0	40.0	49.0
8–14	51.0	48.0	54.0	53.0	41.0	47.0	53.0
15–21	60.0	53.0	49.0	53.0	43.0	53.0	61.0
22–28	58.0	44.0	61.0	57.0	50.0	57.0	54.0
29–Feb. 4	56.0	53.0	57.0	50.0	50.0	55.0	56.0
Feb. 5–11	53.0	51.0	60.0	45.0	58.0	49.0	57.0
12–18	52.0	56.0	52.0	56.0	62.0	53.0	48.0
19–25	51.0	54.0	54.0	58.0	60.0	59.0	59.0
26–Mar. 4	48.0	51.0	54.0	68.0	68.0	56.0	44.0
Mar. 5–11	53.0	52.0	56.0	56.0	68.0	58.0	44.0
12–18	54.0	54.0	54.0	67.0	65.0	65.0	62.0
19–25	50.0	48.0	68.0	63.0	65.0	65.0	62.0
26–Apr. 1	68.0	58.0	62.0	65.0	64.0	59.0	67.0
Apr. 2–8	67.0	54.0	64.0	62.0	69.0	63.0	64.0
9–15	60.0	67.0	59.0	61.0	55.0	69.0	61.0
16–22	68.0	72.0	69.0	69.0	69.0	64.0	72.0
23–29	74.0	73.0	67.0	73.0	69.0	70.0	68.0
30–May 6	76.0	75.0	68.0	69.0	69.0	72.0	73.0
May 7–13	73.0	71.0	78.0	63.0	75.0	73.0	72.0
14–20	72.0	78.0	77.0	69.0	75.0	73.0	72.0
21–27	75.0	80.0	77.0	77.0	79.0	68.0	80.0
28–June 3	80.0	77.0	82.0	79.0	79.0	75.0	77.0
June 3–10	83.0	82.0	80.0	80.0	80.0	81.0	77.0

ON THE DISEASE

Judging from the results obtained under controlled conditions, we would expect initial infection to occur in the field on young growth during periods when free moisture was present on the surface of the leaves between temperatures of 59 and 74.3° F. Initial infection would be most abundant under these conditions when a temperature of about 68 to 70° F. prevailed.

Observations point to the fact that the spores of the pathogene overwinter on the leaves in the old scab spots formed during the preceding season. At least these old scab spots serve as the chief source for the early spring infections. Judging from the many observations made in the field, it appears that the spores of the fungus may also lodge in the scales of the dormant buds and then when these begin to grow in the spring the spores are at hand to produce infection on the young unfolding leaves.

The presence of free moisture on the surface of the leaves is necessary for a rather long interval for the successful germination of the spores to take place and for the germ tube to enter the tissues of the leaf. Even after the hyphae

have entered the tissues, the small and slow amount of growth which the fungus makes necessarily lengthens the period of incubation. The period of initial infection for citrus scab is a question of hours and not minutes. A summary of Fawcett's (*4*) infection experiments shows that the period of incubation is also long, six days to two weeks being required even under optimum conditions for infection.

Primary infection in the spring usually occurs on the unfolding buds, while secondary infection takes place on the young fruits very soon after the petals drop. It is rather unusual under Alabama conditions for the pathogene to attack half-grown leaves or fruits. It is true that scab is found on leaves and fruits of this age, but the length of the incubation period indicates that initial infection takes place while the leaves and fruits are quite small.

The fact that the disease appears on either side of the leaves, precludes the possibility of stomatal infection as no stomata are present on the upper surfaces of Citrus plants. Infection is accomplished, for the most part, by the direct penetration of the epidermis by the fungus hyphae. As the leaves mature the firmer leaf texture would necessarily become less penetrable by the hyphae of the fungus.

Initial infection is not dependent on rapidly growing tissue so much as on the fact that the leaves must be young, whether rapidly growing or not. However, after initial infection takes place, rapidly growing infected tissues produce more malformations and consequently larger and more misshapen leaves and fruits. Any conditions favoring the rankness of the first growth would also contribute to produce larger scab spots.

In figure 1, the minimum, optimum, and maximum temperatures for scab infection, as determined under controlled conditions, are indicated. While these points may not be strictly applicable to what actually occurs in the field, they are sufficiently accurate for the purpose of our discussion. During the early part of some years, the weekly mean temperatures rise above the minimum for infection for short periods, but in most instances, they are followed by sudden drops in temperature. No scab has ever been found during these periods, as the other two conditions for successful infection are not always at hand; namely, sufficient moisture and the presence of young foliage. Temperatures suitable for slight infection occur at different times during the various seasons, as indicated in figure 1. The period during which optimum temperatures for infection prevail is usually from the middle of April through most of May. By the third week in April the temperature during most years reaches the optimum and by the first of June the prevailing temperatures are above the maximum for infection.

If the temperature factor is considered by itself, we should expect the same amount of scab each year, as the optimum temperature for infection is reached at practically the same time. There is only one week or at the most, two weeks' difference in the time required for the temperatures to reach the maximum. We should also expect that the earlier the season the more prevalent scab would be. However, it is exactly the reverse, as will be brought out later.

The presence of moisture over a long enough period for initial infection to take place has been continually stressed as one of the essentials for successful infection. The weekly rainfall, including the number of rainy days per week for the spring season of 1914 to 1920, was tabulated, but no positive correlations between the relative prevalence of scab and the amount or frequency of rainfall could be made. Under Alabama conditions, there is rarely a week in which rain does not occur during the development of the first spring growth. In the majority of cases, when temperatures suitable for infection are at hand, moisture sufficient for initial infection is present. Thus, as far as Alabama is concerned, we can take

it for granted that the rainfall is sufficient during the greater part of the time in which temperatures suitable for infection prevail.

We come now to the most important variable factor and by all means the most difficult to present, namely, the development of the early spring growth and its relation to scab.

In the preceding pages, it has been pointed out that the various types of Citrus plants start growth in the spring when different weekly mean temperatures are reached. These points vary during different years so that in some cases they may occur as much as six weeks apart. Again, not all plants of the same variety start at the same time. Furthermore, during certain seasons all plants may start at practically the same time, or at different times, during years when the weekly mean temperatures are low. The amount of first spring growth and the rapidity with which it matures are also dependent on weather conditions. Thus, all these points must be taken into consideration in duscussing the relative prevalence and susceptibility to scab of various plants.

Any environmental factor or factors which induce a slow or slight spring growth and rapid maturation or late starting of susceptible Citrus trees favor scab escape; while any environmental factor or factors which induce a large amount of spring growth and subsequently slower maturation, especially during the period of optimum infection, favor scab attacks. Thus, we must not only consider the internal factors influencing the development of spring growth of the Citrus trees, but also the external factors which may inhibit or stimulate these processes.

To illustrate, grapefruit plants, as a rule, start rather early in the season and during normal years complete their growth before optimum conditions favorable for scab are at hand. However, plants may go into the winter in a devitalized condition and so start extremely late in the spring and thus escape infection. It is only when environmental conditions are such that the development of the first spring growth coincides with the optimum conditions for scab development that the disease is at all serious on grapefruit plants.

As is well known, Satsumas are generally susceptible to scab. The somewhat higher temperature necessary for the forcing of active growth of Satsuma usually means that it starts later in the season and first spring growth is well developed at the time optimum conditions for infection are at hand.

Until more data are at hand, we can roughly divide the susceptible commercial Citrus species and varieties into three groups, according to their normal development (internal) in the field as follows:

1. Varieties which start early in the season and make a slow and slight growth, which matures rapidly.

2. Varieties which start somewhat later, and produce a larger amount of spring growth, which matures slowly and about the time optimum conditions for infection are at hand.

3. Varieties which start late in the season after the optimum for infection has occurred.

Plants falling in Groups 1 and 3 are generally free from scab, while those in Group 2 are usually easily infected and badly attacked by scab.

External factors, however, play an equally important part in determining the type of spring growth developed and in its scab susceptibility or escape. After a thorough consideration of the numerous factors involved, it is not surprising that no successful infections have been made in the field with this disease. However, it should not be difficult to do when all factors are taken into consideration. Evidently some essential requirements for successful infection in the experiments so far reported have been omitted.

CONDITIONS DETERMINING THE YEARLY PREVALENCE OF CITRUS SCAB

It is a well-known fact that there are epidemic and nonepidemic scab seasons. Thus, in Alabama, the seasons of 1914, 1915, and 1920 were noted as very bad scab years. The season of 1918 was probably the lightest scab year recorded and yet scab was plentiful on some plants in the isolation field as early as March 8. Some scab was present during the years 1916, 1917, and 1919, but these can be classed as nonepidemic years.

Although scab is present during the month of March in some seasons, the weather conditions prevailing during April and May determine largely the relative prevalence of scab. To determine the conditions necessary for an epidemic or nonepidemic year, the seasons of 1916 and 1918 are contrasted with those of 1915 and 1920 (fig. 1).

To facilitate the discussion, it can be said that most plants were in full leaf or were starting growth by the week ending March 4, during 1918. In other words, the mean temperatures for February and March were above the normal for these months, so growth responded very early in the season. Note also that the prevailing temperatures did not approach the optimum for infection until well along in April, by which time the plants had about completed their first spring growth, and so escaped the disease. Let us contrast these conditions with those of 1915. The monthly mean temperature for March was almost 7° below the normal. No temperatures suitable for growth and subsequent infection occurred until the middle of April. The following week, just as the young growth was developing the weekly mean temperature went above the temperature for optimum infection and remained more or less at this point for several weeks. During the first two weeks in May almost 4 inches of rainfall occurred on eight different days. The three conditions essential for infection were thus well supplied so that the year 1915 is noted as the worst scab year on record in Alabama.

TABLE II.—*Monthly mean temperature, precipitation, and number of rainy days at various localities*

MONTHLY MEAN TEMPERATURE

Station	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Annual
Manila, P. I.	77.0	77.2	80.2	82.9	83.5	82.2	80.8	80.8	80.6	80.4	79.0	77.4	80.2
Los Angeles, Calif.	51.0	52.7	60.1	60.4	65.2	70.6	76.3	76.4	72.1	64.2	58.2	53.2	63.0
Mobile, Ala.	49.8	53.2	59.1	66.0	75.6	79.1	80.5	79.7	76.5	61.1	57.5	51.5	66.1
Nagasaki, Japan	42.4	42.4	48.7	57.9	64.4	71.1	78.3	80.0	74.5	64.6	54.9	46.0	60.4

MONTHLY MEAN PRECIPITATION

Manila, P. I.	1.10	0.39	0.67	1.18	4.06	9.80	15.32	13.78	14.53	7.48	5.16	2.28	75.87
Los Angeles, Calif.	2.01	1.98	2.34	0.70	0.35	0.05	0.02	0.14	0.14	0.54	0.77	1.57	10.59
Mobile, Ala.	4.86	5.06	7.17	4.35	4.00	5.95	7.04	6.81	5.02	3.18	3.74	4.57	62.04
Nagasaki, Japan	2.96	3.03	5.04	8.12	7.44	11.10	10.16	7.73	7.09	5.52	3.58	3.64	74.29

NUMBER OF RAINY DAYS

Manila, P. I.	5	3	3	4	9	16	21	21	20	16	12	8	138
Los Angeles, Calif.	6	6	7	4	3	1	0	0	1	3	2	6	40
Mobile, Ala.	11	10	10	8	8	12	15	14	10	6	7	7	121
Nagasaki, Japan	16	13	14	15	14	16	14	13	13	11	12	16	165

While the season of 1916 was somewhat later than that of 1918 and more scab was noted, temperatures below those of 1918 were recorded during most of the development of the spring growth. By the time optimum temperatures for infection occurred growth was completed. However, the amount of growth was smaller and maturation of foliage was more rapid.

The lateness of the season in 1920 was due to the low temperatures which occurred during the first week of March. All the young growth formed before this time was killed back. The mean monthly temperature for March was also below normal. Thus the forcing of growth from new buds was resorted to by the plants, which naturally delayed the development of the first spring growth. However, temperatures very much like those which prevailed during 1915 occurred for the rest of the season, which were very conducive to maximum infection. Thus, during the season of 1920, scab was almost as bad as in 1915.

For practical purposes it appears that a mean monthly temperature below the normal for March in Alabama can be used as an indication of a scab epidemic, while temperatures above the normal for this month and with no freezing temperatures are indicative of a light scab season. The orchard grower then can forecast to some extent light or bad scab seasons from the mean monthly temperature and its departure from normal for March and can regulate his spraying program accordingly, depending on the prevailing conditions.

It might be stated at this point that there is not much use in spraying after the weekly mean temperatures go above 75° F. nor after the first growth period is completed.

WEATHER AND ITS EFFECT ON THE DISTRIBUTION AND PREVALENCE OF CITRUS SCAB

In discussing the distribution of citrus scab it was stated that this disease was rather serious in the Gulf Coast States, Japan, and South China, while scab was not known in the Mediterranean countries, California, and the Philippine Islands, although scab had undoubtedly been introduced into California and possibly the Philippine Islands many times. It was further stated that there were certain factors which prevented the successful propagation of citrus scab in these localities.

To determine just what some of these factors might be, the mean monthly temperature and precipitation and number of rainy days for Manila, P. I.; Los Angeles, Calif.; Mobile, Ala.; and Nagasaki, Japan; were obtained.[3] These data are tabulated in Table II and plotted in figure 2. For the sake of simplicity, in the discussion which follows, all references to the relation of these environmental factors to the development of the host plants will be eliminated. It should be understood, however, that young growth must be present as one of the prerequisites for successful infection.

It can be readily seen that the principal reason why scab is not prevalent in the Philippines is the fact that the mean monthly temperatures for the whole year are above the maximum for infection. Even though scab was introduced on Citrus stock, the fungus could not propagate itself because of this fact. There is no doubt that citrus scab has been introduced into the Philippine Islands on nursery stock from Japan in the past, but owing to the prevailing high mean temperature militating against it, scab has never gained a foothold.

If we follow the temperature curve for Mobile through we find that some scab could occur in March, but that by the end of May temperatures are already above the maximum. The period of optimum infection would be rather short.

[3] Climatic data were kindly supplied by J. Warren Smith, agricultural meteorologist, Weather Bureau, U. S. Department of Agriculture.

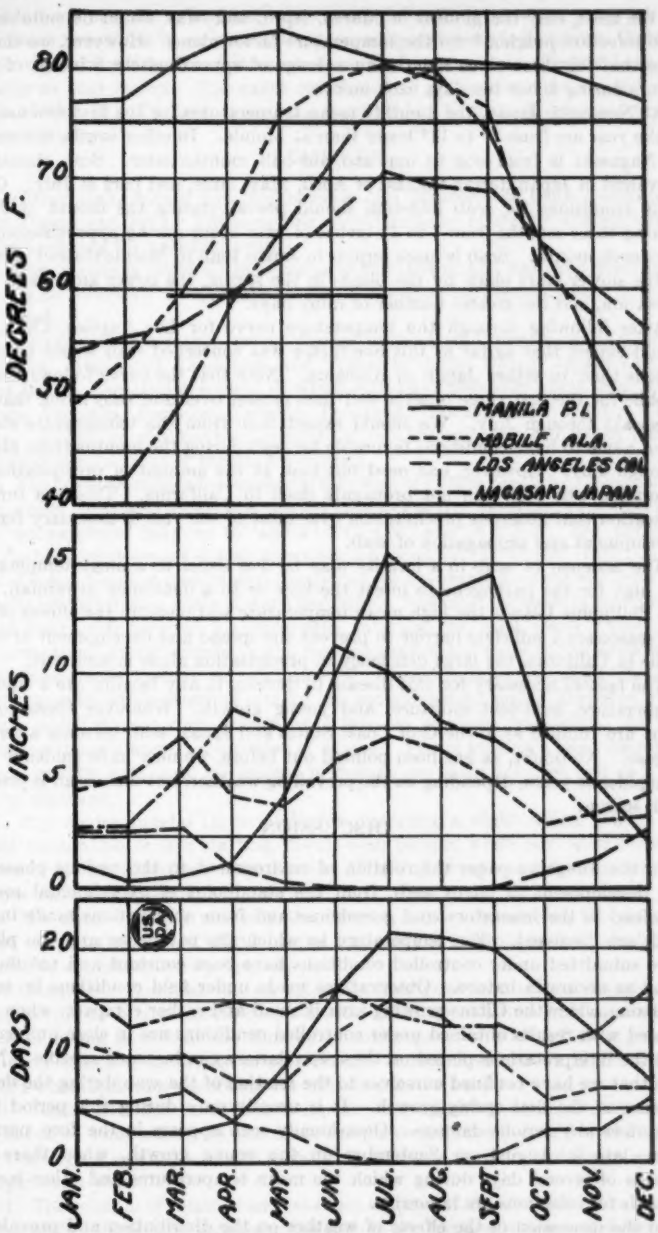


FIG. 2.—Graph showing the monthly mean temperatures, precipitation and number of rainy days for Manila, P. I.; Mobile, Ala.; Los Angeles, Cal.; and Nagasaki, Japan, together with the minimum and maximum temperatures for citrus scab infection

At the most, only the months of March, April, and May would be suitable for scab infection judging from the temperature factor alone. However, we should note that for these three months an average of approximately 5 inches of rain occurs during about ten days each month.

At Nagasaki, Japan, the monthly mean temperatures for the first five months of the year are from 8° to 11° lower than at Mobile. In other words, the season at Nagasaki is from one to one and one-half months later. Scab should be prevalent in Japan during the last of April, May, June, and part of July. Optimum conditions for scab infection should prevail during the month of June. During these months from 7 to 11 inches of rain occur during approximately 15 days each month. Scab is more serious in Japan than in Mobile district because of the slower start made by the plants in the spring, the larger amount of precipitation, and the greater number of rainy days.

After following through the temperature curve for Los Angeles, Calif., we would expect that as far as this one factor was concerned scab would be more serious than in either Japan or Alabama. Note that the curve follows that of Mobile for the first three months and then crosses over and runs along that for Nagasaki through July. We should expect then from the temperature standpoint alone to have conditions favorable for scab during the months from March through July. However, one need but look at the amount of precipitation to determine why scab can not propagate itself in California. This is a further indication that generous precipitation over most of the year is necessary for the development and propagation of scab.

The absence of scab in a locality may be due either to a mean temperature too high for the pathogene to infect the host or to a deficiency in rainfall. In the Philippine Islands the high mean temperature and possibly the effects of the dry season are a sufficient barrier to prevent the spread and development of scab, while in California the large deficiency in precipitation alone is sufficient.

The factors necessary for this disease to develop in any locality are a suitable temperature, sufficient moisture, and young growth. Wherever these conditions are fulfilled as in the Gulf Coast States and Japan, scab becomes a serious disease. No doubt, as has been pointed out before, we may have epidemic and nonepidemic years, depending on the prevailing weather, but some scab is present each season.

DISCUSSION

In the foregoing pages the relation of environment to the various phases of the development of citrus scab, from the standpoint of experimental results obtained in the laboratory and greenhouse and from observations made in the field, are discussed. The temperature to which the pathogene and the plants were submitted under controlled conditions have been constant and not fluctuating as occurs in nature. Observations made under field conditions in south Alabama, where the Citrus-growing area is small and rather compact, when correlated with results obtained under controlled conditions are in close uniformity and the interpretations placed on these correlations can be made specific. Note also that we have confined ourselves to the relation of the scab during the development of the first spring growth. It is usually only during this period that scab does any serious damage. Occasionally scab appears in the fore part of June, late in August, or September on the young growth, when there are periods of several days during which the mean temperatures and other factors suitable for infections are favorable.

In the discussion of the effects of weather on the distribution and prevalence of scab in the other Citrus-growing regions we have tried to point out in a general way that there are certain types of weather which either favor or inhibit the

development of scab. Obviously one could not without a close study of the conditions prevailing in an entire Citrus-growing region state positively that the whole region would be free from scab, from the weather data obtained from one locality in that region. The exact correlation of environment to the development of scab in a Citrus-growing area must be left to trained observers present in that locality.

SUMMARY

1. Citrus scab, caused by *Cladosporium citri* Massee, has been encountered with frequency during citrus-canker investigations made by the writers in Alabama during the past four years.

2. The disease is probably indigenous to the Orient, and has been distributed directly or indirectly from Japan to the Citrus-growing regions where it is not prevalent.

3. Citrus scab is a rather serious disease in the Gulf Coast States of Florida, Alabama, Mississippi, Louisiana, and Texas; in South China, Japan, and the West Indies. Scattering reports of citrus scab have also been made from South Africa, Australia, Formosa, Yucatan, Paraguay, Hawaii, India, and the Canal Zone. As yet, no scab has been reported from California, the Philippine Islands and the Citrus regions of the Mediterranean.

4. The three essentials for successful infection of Citrus plants by *Cladosporium citri*, under controlled conditions, are the presence of free moisture, young growth, and temperatures between 15° and 23.5°C. (59° and 74.3°F.). The optimum for the best development of scab appears to be about 20°–21°C. (68°–70°F.).

5. Under Alabama conditions, temperatures favorable to optimum infection usually prevail during parts of April and May. Sufficient moisture is generally at hand during this interval for successful infection to take place. The most important and variable factor is the development of the early spring growth.

6. Susceptible species or varieties of Citrus making a slight or slow spring growth which matures rapidly are not subject to scab. Likewise, those plants which do not start growth until late are also free from scab. In both instances it is apparently a question of escape. Plants making a large amount of spring growth, with slow maturation of foliage are easily infected and as a rule badly attacked.

7. Any environmental factor or factors including a slight spring growth and rapid maturation or late starting, favors scab escape; while any environmental factor or factors inducing a large amount of spring growth and slow maturation, favors scab susceptibility.

8. We must consider not only the internal factors influencing the development of spring growth of Citrus plants, but also the external factors which may inhibit or stimulate these processes.

9. Conditions essential for an epidemic scab year in Alabama depend to a large extent on a late season, coupled with sufficient moisture and the development of spring growth at the time optimum temperatures for infection prevail. An early season is favorable to scab escape, in that first growth is about completed when optimum conditions for infection are at hand.

10. Under Alabama conditions it appears that a light or bad scab year can be predicted to some extent by the mean temperature prevailing in March; a temperature below normal indicates a bad scab season; a temperature above normal, a light scab year.

11. The relation of weather and its effect on the distribution and prevalence of citrus scab is shown and the absence of scab in certain localities is correlated with either a mean temperature too high for infection or a deficiency in rainfall.

LITERATURE CITED

(1) FAWCETT, H. S.
1912. CITRUS SCAB. Fla. Agr. Exp. Sta. Bul. 109, p. 51–60, illus.

(2) ———
1915. CITRUS DISEASES OF FLORIDA AND CUBA COMPARED WITH THOSE OF CALIFORNIA. Calif. Agr. Exp. Sta. Bul. 262, p. 149–211, illus.

(3) ———
1916. CITRUS SCAB. Phytopathology 6: 442–445.

(4) ———
1921. SOME RELATIONS OF TEMPERATURE TO GROWTH AND INFECTION IN THE CITRUS SCAB FUNGUS CLADOSPORIUM CITRI. Jour. Agr. Research 21: 243–253.

(5) LEE, H. A.
1918. EARLY OCCURRENCE OF CITRUS SCAB IN JAPAN. Phytopathology 8. 550.

(6) PELTIER, G. L.
1920. INFLUENCE OF TEMPERATURE AND HUMIDITY ON THE GROWTH OF PSEUDOMONAS CITRI AND ITS HOST PLANTS AND ON INFECTION AND DEVELOPMENT OF THE DISEASE. Jour. Agr. Research 20: 447–506.

(7) REINKING, O. A.
1919. DISEASES OF ECONOMIC PLANTS IN SOUTHERN CHINA. Philippine Agr. 8. 109–136, illus.

(8) ———
1921. CITRUS DISEASES OF THE PHILIPPINES, SOUTHERN CHINA, INDO-CHINA, AND SIAM. Philippine Agr. 9. 121–180, illus.

(9) SWINGLE, W. T., and WEBBER, H. J.
1896. THE PRINCIPAL DISEASES OF CITRUS FRUITS IN FLORIDA. U. S. Dept. Agr., Div. Veg. Physiol. and Path. Bul. 8, p. 1–42, illus.

A STUDY OF ENSILING A MIXTURE OF SUDAN GRASS WITH A LEGUME[1]

By P. A. WRIGHT, *Assistant Chemist*, and R. H. SHAW, *Chemist, Research Laboratories, Dairy Division, Bureau of Animal Industry, United States Department of Agriculture*

OUTLINE OF EXPERIMENT

This paper reports a study of ensiling a silage crop high in protein and low in carbohydrates, mixed with one low in protein and high in carbohydrates, to determine whether such a mixture makes better silage than the same crops ensiled separately.

Two legumes, soybeans and cowpeas, were the high-protein crops used, and Sudan grass was the low-protein, high-carbohydrate crop.

Experimental silos were filled with each of the crops alone, with a mixture of Sudan grass and soybeans, and with a mixture of Sudan grass and cowpeas. The silage was rated on the basis of keeping quality, palatability, chemical composition, and loss in food constituents.

PREVIOUS INVESTIGATIONS

It is a common practice in many sections of the country to mix together two different silage crops, one as supplement to the other, supplying in greater amount what the other lacks in certain feed constituents. The usual combination is a material rich in fermentable carbohydrates but low in protein, like corn; with one rich in protein, like the legumes. Typical combinations are oats and peas, corn and soybeans, sweet sorghum and alfalfa.

Sudan grass, soybeans, and cowpeas have all been ensiled, both alone and in combination with other crops. Sudan grass has been ensiled alone by Francis and Friedemann[2] at the Oklahoma Experiment Station; Sudan grass, soybeans, and cowpeas have been ensiled alone with success at the Missouri Experiment Station by Eckles,[3] and Sudan grass has been ensiled with rape with satisfactory results by Lamb and Evvard[4] at the Iowa Experiment Station. Soybeans have been ensiled with corn frequently, but most recently by Dvorachek and Mason[5] at the Arkansas Experiment Station, who state that a silage 10 per cent more valuable was obtained by drilling in soybeans with corn and ensiling them together than by using corn alone.

---

[1] Received for publication April 18, 1924.

[2] FRANCIS, C. K., and FRIEDEMANN, W. G. SUDAN GRASS SILAGE. Okla. Agr. Exp. Sta. Bul. 115, 8 p., illus. 1917.

[3] ECKLES, C. H. LEGUMES, SUDAN GRASS AND CEREAL CROPS FOR SILAGE. Mo. Agr. Exp. Sta. Bul. 162, 25 p. 1919.

[4] LAMB, A. R., and EVVARD, J. M. RAPE AS MATERIAL FOR SILAGE. Jour. Agr. Research. 6: 527-533. 1916.

[5] ARKANSAS AGRICULTURAL EXPERIMENT STATION. BETTER SILAGE BY ADDING A LEGUME. Ark. Agr. Exp. Sta. Bul. 181: 60-61. 1922.

EXPERIMENTAL WORK

FIELD METHODS

The Sudan grass, soybeans, and cowpeas were sown on June 3, in separate plots of less than an acre, on land previously used for corn at the United States experiment farm at Beltsville, Md. The first two made good growth, but the stand of cowpeas was very thin and spindling. They were all harvested September 2, when the Sudan grass was in the dough stage and the soybeans and cowpeas were well podded. All the Sudan grass and the part of the soybeans which was ensiled with it were hauled to the cutter fresh; but the remainder of the soybeans, which was ensiled alone, and all the cowpeas were allowed to wilt before being hauled from the field.

The experimental silos used were wooden tubs 2 feet in diameter and 4 feet deep. They were provided with wooden followers fitting as closely as possible within the silo walls. Pressure of 100 pounds per square foot, to approximate the pressure within a large silo, was obtained by means of a lever and weight applied to the center of the follower. The silo was filled to within 16 inches of the top, and a piece of cheesecloth was then carefully spread over to make a complete separation between the portions of silage below and above. The portion below the cheesecloth was called the experimental portion. The remainder of the silo was then packed with the same kind of silage material, another piece of cheesecloth was spread on, and the follower, lever, and weight were adjusted in place.

Five of these silos were used. They were filled as follows: (1) Sudan grass alone; (2) Sudan grass and soybeans, half-and-half; (3) wilted soybeans alone; (4) wilted cowpeas alone; (5) Sudan grass and wilted cowpeas, half-and-half mixture. Samples were taken at the time of filling for laboratory analyses.

Three months after ensiling, on December 3, all five silos were opened. The top portions down to the cheesecloth separators were weighed and discarded. The remainder, or experimental part, in each silo was weighed, thoroughly mixed, and a portion of the well-mixed lot taken as a sample for laboratory analysis. After the laboratory samples were taken, the remaining portions of the silages were used as material to conduct comparative palatability tests.[6]

METHODS OF ANALYSIS

A 1-kilogram subsample from a thoroughly mixed field sample of each kind of silage was dried in a steam-heated drying closet at about 55° C. to air-dry weight. These subsamples were then ground in an electric pulverizing mill so as to pass a 40-mesh sieve. Moisture, total protein, albuminoid protein, ash, crude fiber, and ether extract were determined on the finely ground sample according to methods outlined in "Methods of Analysis" by the Association of Official Agricultural Chemists.[7] Moisture was found by adding the weight of moisture lost in obtaining the air-dry sample to that lost on drying the air-dry sample, and calculating as a percentage of the original moist material.

[6] Acknowledgment is made to H. T. Converse for suggesting and conducting the palatability test and summarizing the results.

[7] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. As compiled by the Committee on Revision of Methods. Revised to Nov. 1, 1919. p. 71–90. 1920.

TABLE I.—*Loss or gain in weight of experimental portion of silage*

Kind of silage	Experimental portion		Loss (−) or gain (+)
	Put in	Taken out	
	Pounds	*Pounds*	*Per cent*
Sudan grass alone	198.0	198.5	+0.25
Sudan grass and soybeans	198.0	196.0	−1.0
Soybeans alone	207.0	209.5	+1.2
Cowpeas alone	246.0	247.0	+0.4
Sudan grass and cowpeas	185.0	184.5	−0.3

DISCUSSION OF RESULTS

There was very little apparent change in weight on the experimental portion of any of the silos. Except in one case, that of the Sudan grass alone, all the experimental portion was excellent silage, and in most cases some of the filler portion also. The Sudan grass alone did not pack well, so that not only was all the filler spoiled, but also about 10 pounds of the experimental portion were moldy. So far as one could judge by taste and smell of the silage, that in one silo kept as well as another, or nearly so. In general appearance, however, the mixtures made much better silage than the Sudan grass. On the other hand, the mixtures, while yielding excellent silage, did not seem to yield any better silage than the legumes alone. As suggested by Eckles,[8] the Sudan grass and the cowpeas were allowed to wilt before being cut for the silo, and, as he found, a perfectly normal and very palatable silage was produced.

PALATABILITY

The palatability test conducted with the different silages furnished a very interesting and practical basis of comparison.

If the palatability of the silages is to be judged by the way in which the cows ate them at the start, the Sudan grass had little influence, if any, in either increasing or decreasing the palatability of the legumes, even though the Sudan-grass silage alone was quite unpalatable. The final conclusion reached as a result of the palatability test was that the soybean silage was very slightly more palatable than the cowpea silage; that the legumes were very slightly more palatable than the mixtures; that there was no appreciable difference between the mixtures; and that Sudan-grass silage alone was far less palatable than any of the other silages.

CHEMICAL COMPOSITION

Table II gives the composition of the silage crops as they were put into the silo and of the silages as they were taken out.

These data show that dry matter does not vary much between silos except in the case of the soybeans alone, which is probably due to wilting. As might be expected, the percentages of total and albuminoid proteins are considerably greater in the mixtures than in the Sudan grass and less than in the legumes. Ash seems to be highest in the cowpea silage and lowest in the Sudan grass alone. Crude fiber does not vary much between samples except in the case of cowpeas alone, where it is considerably lower. Ether extract does not vary much except with the soybeans alone, where it is somewhat lower than in the other silage. Nitrogen-free extract is highest in the Sudan grass and lowest in the soybeans alone, though it must be said the differences are not remarkable.

[8] ECKLES, C. H. LEGUMES, SUDAN GRASS AND CEREAL CROPS FOR SILAGE. Mo. Agr. Exp. Sta. Bul. 162, 25 p. 1919.

TABLE II.—*Composition of silages as put into and taken out of silos*

Kind of silage	Dry matter in moist material		Water-free basis											
			Total protein		Albuminoid protein		Ash		Crude fiber		Ether extract		Nitrogen-free extract	
	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out	In	Out
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Sudan grass alone	30.3	28.9	6.0	6.4	5.3	4.5	5.5	6.1	34.0	35.2	1.5	1.9	53.0	50.4
Sudan grass and soybeans	30.3	30.1	11.0	11.3	9.0	7.1	6.8	7.5	31.6	34.0	1.4	2.3	49.3	44.9
Soybeans alone	36.3	34.8	16.7	17.6	12.7	9.8	6.7	8.3	30.5	32.7	1.2	2.0	44.9	39.8
Cowpeas alone	31.8	31.0	16.0	15.9	13.4	9.9	7.7	8.8	23.8	27.1	1.5	2.6	51.0	45.6
Sudan grass and cowpeas	33.3	30.6	11.8	12.3	9.8	8.9	7.1	8.1	29.9	30.6	1.7	2.5	49.6	46.6

Table III gives the percentage of loss or gain in different feed constituents of the silage, based on the weights of each put in and taken out of the silo. Gain in weight of any of the feed constituents can be explained only by down wash from the filler portion of each silo of portions of these constituents. The loss or gain of weight in moisture and dry matter as shown in Table III is not remarkable in any of the silages. Sudan grass and cowpea mixture shows the greatest loss of dry matter, followed by Sudan grass alone. As usual, in silage there is a large loss of albuminoid protein due apparently to the breaking down of the more complex protein into simpler nitrogen compounds. These simpler nitrogen compounds are generally considered to have less feed value than the original albuminoids. The losses of albuminoids are larger in each legume silage alone than when Sudan grass was ensiled with this legume. This would indicate that a considerable loss of feeding value was prevented by the presence of the Sudan grass.

The ash shows an unusual gain in all cases, from 5 per cent in the Sudan grass silage to 21 per cent in the soybean silage, and 23 per cent in the Sudan grass and cowpea mixture. These gains correlate very well with the gains in amount of moisture, and may be due to a down wash of soluble ash from the filler portion of the silage which lay above the experimental portion. Crude fiber shows a gain of 9 per cent in the cowpea silage and a gain of nearly 6 per cent in the Sudan grass and soybean mixture, while in Sudan grass silage and the Sudan grass and cowpea mixture, losses are indicated. Ether extract shows gains of from 7 per cent in the cowpea silage to 54 per cent in the Sudan grass and soybean mixture. There is a loss of nitrogen-free extract in every silage, varying from 9 per cent in Sudan grass silage to 15 per cent in soybean silage. The loss of nitrogen-free extract and therefore of feeding value is somewhat larger in the soybean silage than in the soybean and Sudan grass mixture. No such difference is shown between the cowpea silage and the cowpea and Sudan grass mixture.

TABLE III.—*Loss (−) and gain (+) in weight of silage constituents as put into and taken out of silos*

Kind of silage	Green material	Moisture	Dry matter	Total protein	Albuminoid protein	Ash	Crude fiber	Ether extract	Nitrogen-free extract
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Sudan grass alone	+0.25	+2.2	−4.3	−1.8	−19.3	+5.2	−1.2	+23.9	−9.0
Sudan grass and soybeans	−1.0	−0.7	−1.8	+1.4	−22.7	+8.5	+5.8	+54.0	−10.5
Soybeans alone	+1.2	+3.7	−3.2	+1.7	−25.4	+21.0	+3.9	+52.2	−15.0
Cowpeas alone	+0.4	+1.5	−1.9	−2.3	−27.7	+11.1	+9.1	+7.3	−12.3
Sudan grass and cowpeas	−0.3	+3.6	−8.1	−4.0	−17.0	+23.0	−6.0	+33.5	−13.9

CONCLUSIONS

There was little difference in keeping quality among the silages studied. The legumes ensiled alone produced as normal a silage as the mixtures. Sudan grass produced a silage which appeared as normal as the others, but as it did not pack so firmly it yielded a larger amount of spoiled silage.

The legumes were slightly more palatable than the mixtures, and the Sudan grass was very much less palatable than the rest of the silages.

Outside of the Sudan grass silage, which was very low in protein, and soybean silage, which was low in nitrogen-free extract, the differences in chemical composition did not indicate any marked superiority of one silage over another. However, if there was any silage better than another on the basis of chemical composition, it was the cowpea silage. The mixtures were practically as good as the legumes, except in protein content, but no better.

The legumes showed a greater actual loss of albuminoid protein and therefore of feeding value than the mixtures. Soybean silage showed a greater loss of feeding value in nitrogen-free extract than the soybean-Sudan grass mixture. Sudan grass alone showed the least loss of these valuable feed constituents.

It does not seem necessary, from this study, for the production of good silage to ensile together a crop high in protein with one high in carbohydrates. The crop carrying a large percentage of carbohydrates usually takes care of itself in the silo; and the high-protein crop, if wilted so as to contain the proper moisture content, also produces a satisfactory silage.

BACTERIAL LEAFSPOT OF DELPHINIUM[1]

By MARY K. BRYAN

Assistant Pathologist, Laboratory of Plant Pathology, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The leafspot of Delphinium (larkspur), commonly known as the "Black spot," or "Black disease," is a very destructive disease, widespread throughout the northern United States, more particularly in the North Atlantic States. Although this decorative plant has been eliminated from the gardens of some large estates because of its ravages, the disease has received scant attention from pathologists.

The only references in pathological literature are brief notes by Dr. Erwin F. Smith. In "Science," for March, 1904,[2] he gave a very brief description of the causal organism which he had isolated, proved pathogenic by inoculations and named *Bacillus delphinii*. Cultures were allowed to die before adequate cultural work was done, and although the organism was again isolated in 1907, pressure of other work crowded out its further study. The spots are figured by him in "Bacteria in Relation to Plant Diseases,"[3] and in the same volume, page 92, it is stated to be one of the diseases transmitted through stomata and waterpores. He later[4] refers again briefly to the subject, as follows:

"The spot disease of Delphinium (Vol. I, fig. 127) is another malady in which infection takes place readily through the unbroken leaf-surface and stem-surface, i. e., through stomata. The disease has been obtained a number of times during the last seven years by placing the bacteria in water and spraying this upon the plants. The leaf-serratures also blacken in this disease, and here infection probably occurs through the groups of water pores situated on their apex."

In 1920 characteristically spotted leaves were received from Woodstock, N. Y., from which isolations were made of a white bacterial organism. These and subsequent isolations from various sources have been used for the study here presented.

HOST PLANTS

This disease has not been reported on any plant except larkspur, being most destructive on the choice hybrid delphiniums. A few small but definite infections have been produced on Aconite by rubbing the inoculum on the lower surface of leaves, but no natural infections have been reported on this plant. No infections were obtained by repeated inoculations on cultivated varieties of Ranunculus and Aquilegia.

GEOGRAPHICAL DISTRIBUTION

Specimens have been received from points in Maine, New Hampshire, Massachusetts, Connecticut, New York, Long Island, Pennsylvania, Illinois, and in one case from Portland, Oreg. One correspondent wrote in 1922, "I have just

[1] Received for publication Jan. 23, 1924.

[2] SMITH, E. F. BACTERIAL LEAF SPOT DISEASES. Science 19: 417. 1904.

[3] SMITH, E. F. BACTERIA IN RELATION TO PLANT DISEASES. v. 1, fig. 127. 1905.

[4] SMITH, E. F. BACTERIA IN RELATION TO PLANT DISEASES. 2: 61-62. 1911.

returned from a trip and find that the delphinium all the way from Ridgefield, Conn. out to Chicago, Ill., is badly affected by the 'black disease.'" A delphinium grower and breeder in California reported in 1923 that the "Black disease" which gives so much trouble in the East had not as yet appeared or seemed not to be known on the West Coast.

Leaves from Castine, Me., received early in July, 1922, were 23 cm. across and came from plants 3 meters high. They were thickly covered with the largest spots observed, i. e., 1 to 2 cm. in diameter (Pl. 1, B). Probably the cool, moist climate in this location which was a large factor in the vigor of these plants also increased the virulence of the disease. On less vigorous plants the spots are usually smaller (Pl. 1, A). No specimens have been received from farther south than Pennsylvania, and inoculations in Washington, D. C., where the climate does not favor this plant, while giving numerous typical infections, have never produced spots more than a few mm. wide (Pl. 1, C). During the heated summer term it has been found impossible to produce artificial infections in Washington, D. C., except in the event of a cool, rainy spell.

APPEARANCE OF DISEASED PLANTS

The leafspots are very striking in appearance. On the upper surface they form tarry black areas of irregular shape and size that may reach a diameter of 2 cm. on large vigorous leaves (Pl. 1, B). On the lower surface they are brown and the smaller ones do not yet show through to the upper side. Old spots lack the water-soaked edges that so frequently accompany other bacterial spots, although in very young stages water-soaking is present and is the first indication of infection observable under a hand lens. Although the lower surface of spots may appear sunken, the upper surface usually appears to be raised slightly and, in many spots, there is a tendency to concentric rings strongly suggesting fungus infection (Pl. 2, D and E). Such spots were repeatedly sectioned to make sure that no mistake was being made, but in every case bacteria oozed out in vast quantities, while no fungus was observed and cultures of *Bacterium delphinii* were obtained when poured plates were made. Spots resulting from pure culture inoculations also often showed this zoning, which has since been noted in bacterial spots on other plants, notably tobacco.

Spots may occur on any part of the leaf blade, the result of stomatal infection, and are also common on the leaf tips where they make their entrance through the large water-pores (Pl. 2, A, B, and C). When infection takes place on young leaves, and especially in the deep sinuses, distortion usually results from the failure of the diseased areas to keep pace with the growing healthy tissue. Flower buds are also occasionally attacked, becoming black-spotted and distorted, but this should not be confused with the common distortion of buds and young leaves caused by mite infestation where a blackening of the very young tissues often occurs in severe cases. This is a continuous blackening instead of a spotting and no bacteria are present. The bacterial spot occurs also on petioles and stems. In later stages of the disease the spots may coalesce, forming large black areas, involving in some cases almost the entire blade of the leaf.

HISTOLOGY OF DISEASED LEAVES

The bacteria enter the leaf through the stomata of the lower epidermis or through the water pores at the tips of the serratures. As the spongy parenchyma just beneath this epidermis is very loose, it is difficult to demonstrate stomatal infection in its earliest stages, since the bacteria scatter so readily on entering. Also, unless sections are made early enough the infection spreads so rapidly as

to obscure any evidence of the point of entry. Material fixed 24 hours after spraying gave no sections suitable for photographic demonstration although the spots were water-soaked and scattered bacteria were found under the stomata in microtome sections. After 48 hours, however, very good sections were obtained showing bacteria in the intercellular spaces under the stomata chiefly lying on the cell walls, singly or in oval masses (Pl. 3, A and B). At this stage the spot shows as a minute water-soaked point on the lower surface, to be seen only under a lens. In microtome sections the bacteria are confined to the loose parenchyma. Cells in the vicinity of the invaders often show on their walls what appear to be extrusions of cell sap. These are hemispherical bodies which stain very deeply (Pl. 4, A). Later the palisade tissue is invaded and killed and the lesion penetrates to the upper surface as a black spot involving the whole thickness of the leaf. At this stage of infection the bacteria lie on the walls of the cells and crowd the intercellular spaces.

ISOLATIONS

Poured plates were made in this laboratory in 1903 from spotted leaves from New York and Massachusetts and again in 1907 from Massachusetts. In each case a white organism was obtained with which successful infections were secured on delphiniums. On account of the small amount of cultural work done at that time no adequate comparison can be made with isolations of 1920–1923. Descriptions and photographs of agar plate colonies agree with those of later date but in other cultural characters there is disagreement, not only between these early isolations and those of 1920–1923 but also among the several early isolations themselves, and it is apparent that some of the early cultural work was not fully checked up, since striking inoculations were obtained in 1903 proceeding from subcultures of single poured-plate colonies of what was undoubtedly this organism.

Isolations have now been made from typical infected plants from all of the States mentioned under "Geographical distribution." All gave colonies apparently of the same white organism. Comparative cultures have shown these to be alike in all important points.

In several cases where material was not in good fresh condition when received a yellow organism appeared on the plates in numbers about equaling the white colonies and, in one case, only a yellow organism was obtained although three separate platings were made from typical spots. This was of a saprophytic type frequently appearing on plates poured from many species of plants when not in perfectly fresh condition, and it failed to produce any infections when the inoculum was either sprayed or rubbed on the lower surface of delphinium leaves.

INOCULATIONS

Successful inoculations have been made in the hothouse and out of doors, on young and on mature plants, both in cages where moisture could be maintained and also without cover. Infections obtained on young plants in the hothouse never increased much in size (1 or 2 mm. wide) and few secondary infections were found. Reisolations were made from hothouse inoculations of 1920 and used for inoculations in the hothouse in 1921 and 1922 with similar results. Outdoor inoculations in the summer of 1921 failed, that is only a very few slow-growing infections were secured, probably because of the late start made and supervening dry, hot weather.

On May 31, 1922, vigorous young seedling plants of hybrid delphiniums, which were set out of doors May 1 and were just beginning to send up blossom stalks, were sprayed with subcultures from an isolation of March 29, 1922, from a hot-

house infection. It was a cool cloudy day with threat of rain and the plants were left uncovered. They were sprayed with the organism on two successive cloudy days, and then a three days' continuous rain set in. Nine days after the first spraying the leaves were thickly speckled with infections, especially those inoculated from one colony, individual spots being as much as 2 mm. wide and some confluent areas as large as 1 cm. across. These spots were black, surrounded by yellow areas on some leaves while on others (older mature stem leaves) no yellowing occurred. Poured plates from such spots gave pure cultures of the white organism used for inoculation. The weather then turned very warm, the spots did not increase materially in size and later attempts at inoculation failed, both with reisolations from these leafspots and with the colonies used to produce them. It seems evident that climatic conditions, both cold and moisture, play a large part in the success of inoculations. High temperatures inhibit, and moist cool conditions greatly favor the development of the disease.

During April and May, 1923, several sets of inoculations were made out of doors on delphinium plants that had wintered in the garden. An isolation of 1921 which had produced the infections shown on Plate 4, A and B, and isolations of 1922 were used. Although conditions of moisture and temperature were favorable, very few spots were produced and some inoculations failed entirely.

Numerous typical infections were secured with fresh isolations from delphinium leaves received from Maine on July 10, 1923, by spraying and rubbing the inoculum on plants in a shaded bed (Pl. 1 C). Inoculations were made as soon as subcultures were obtained. Inoculations with the same cultures on plants in the open garden failed. A 24-hour rain at the time of inoculation was followed by hot, dry weather.

THE ORGANISM

MORPHOLOGY

The bacterium causing this disease is a short rod with rounded ends, 1.5 to 2μ long by 0.6 to 0.8μ broad, stained from 24-hour agar cultures. It is actively motile both when floated out from sections and in cultures. The flagella are bipolar, their number varying from 1 to 6 (Pl. 4, B). Rods occur singly or in pairs in most cultures but short chains have been observed in the pellicle of young (3-day) bouillon cultures, in old litmus milk cultures, and in the pellicle on cultures in Fermi's solution. In the sediment weighing down the pellicle of 4-day old bouillon cultures there occur, mixed with rods, many spherical bodies with greater diameter than the rods (Pl. 4, C). The fact that they frequently occur in pairs suggests a clumping of the cell contents of paired rods of which the cell wall has disintegrated or become resistant to the stain. Large numbers of these spherical forms are found in the sediment in the bottom of the tube. These bodies are not spores, since they are easily stained and are not resistant to heat. No spores have been observed. Capsules occur in the pellicle on Fermi's solution.

REACTION TO STAINS

The bacteria stain readily with carbol fuchsin and gentian violet, are not acid-fast and do not stain by Gram.

COLOR PRODUCTION

The bacterial growth is white on all media used, but a brown color forms in Dunham's solution 2 weeks old and sometimes in Cohn's solution (2 months). Very pronounced blue-green fluorescence is produced in Fermi's solution and in Uschinsky's solution and also in beef bouillon, beef agar, and beef gelatin.

CULTURAL CHARACTERS

BEEF AGAR PLATES.[5]—Colonies are visible on the second day and by the third day in thin sown plates are 3 to 5 mm. wide, round, white, transparent, opalescent, slightly convex, sometimes slightly umbonate, smooth shining, finely granular. Young colonies show coarse cross-hatching by oblique transmitted light (Pl. 4, F and G) and sometimes these internal cross-hatchings persist in the older colonies at summer temperatures but usually they give place to internal concentric markings (Pl. 4, E). Buried colonies are lenticular. When older, the colonies may reach a diameter of 8 mm. The consistency is sometimes slightly viscid. Occasionally a ring of irregular opaque areas or radiating lines may appear in the colonies (Pl. 4, D) and old colonies may become slightly lobed. Plates from old litmus milk and from old bouillon cultures have given very convex colonies with depressed centers which, as they enlarge, have a wrinkled surface. After return to favorable cultural conditions, transfers from these colonies again gave normal colonies on agar plates.

BEEF AGAR SLANTS.—Growth is thin, white, smooth shining, transparent, opalescent, with internal wavy markings and entire margins. Growth does not cover the surface but tapers upward from a width of 3 to 6 mm. There is considerable white precipitate in the V. Numerous small crystals form beneath the growth and the agar becomes green.

POTATO CYLINDERS.—The moderate, thin, spreading growth on potato cylinders is dirty white becoming pale tan colored, with a slimy consistency; the potato begins to gray in 24 hours and in a few days is gray throughout. There is no distinct odor. Starch is only partially digested, i. e., a purple color is produced by adding iodin to the crushed potato cylinders on which the organism has been growing.

STARCH AGAR PLATES.—Smears were made on starch agar poured plates from young agar cultures. When tested after 7 days by drenching the surface of the plate with iodin solution an area 5 to 8 mm. wide surrounding the growth was purple in color but there was no area of complete starch destruction.

WHEY AGAR.—On whey agar titrating +8 or P_H 7.4 colonies are very convex, creamy white, opaque, round with entire margins, not opalescent, with internal concentric markings in the margins and in thin sown plates are 5 to 7 mm. wide when 10 days old.

BEEF BOUILLON.—Clouding occurs near the surface in 24 hours with a delicate pellicle which falls readily in fragments on being disturbed. Undisturbed cultures 2 days old are well clouded throughout and have a heavy pellicle, borne down in the center with a mass of white growth, giving the appearance of gelatin liquefaction (Pl. 4, H) when viewed from the side, but from the top showing a nail-head of white in the center of the membranous pellicle. This pellicle falls readily as a whole on shaking. Disturbed cultures re-form their pellicle quickly when young but not after the cultures are 6 or 8 days old. Blue-green fluorescence begins at the top in young cultures. Old cultures are green throughout.

BEEF GELATIN PLATES.—In +10 (P_H 7.2) beef gelatin, kept at 20 to 22° C., colonies on the second day are minute white spots lying in shallow pits of liquefaction several times their width. By the fourth to the sixth day colonies may reach a diameter of 1 cm., clouding the liquefied gelatin uniformly except for a heavier mass of growth at the center.

BEEF GELATIN STABS.—Liquefaction begins on the second day (20 to 24° C.) and is napiform, becoming stratiform. Liquefaction is complete in two to three

[5] All beef media used were made with beef infusion plus 1 per cent Difco peptone. Titrations expressed by + and − are according to Fuller's scale.

weeks with deep green fluorescence. Cultures kept one year on culture media liquefy gelatin very slowly.

MILK.—Milk clears uniformly without coagulation, beginning about the seventh day. Cultures one month old are translucent and honey yellow. No crystals are formed at this time but an occasional culture held for three months has a few small masses of tyrosin crystals. The milk at this age is tawny yellow and translucent (Ridgway XV)[6] and the precipitate has become yellow.

LITMUS MILK.—Litmus milk begins to blue on the third day from the top downward, then clears without coagulation. The cream rim when present is reddened. Reduction of the litmus begins in 10 days and is complete in 20 days. A copious white precipitate is formed. The blue color returns in 2 to 4 weeks. After 3 months the milk is still blue and translucent but the precipitate has become yellow.

METHYLENE BLUE MILK.—Reduction begins on the second day and is complete in 3 to 5 days. No coagulation occurs.

COHN'S SOLUTION.—Weak clouding is visible within 4 or 5 days and increases slowly for 5 or 6 weeks when the cultures are moderately clouded. After 3 months' growth there is a small amount of white precipitate in the bottom of the tube with numerous small crystals.

USCHINSKY'S SOLUTION.—Within 24 hours a faint clouding may be seen in the upper part of the culture. By the second day a delicate pellicle has formed with blue-fluorescence just below it in the upper 2 or 3 mm. and by the fifth day clouding is heavy with a heavy pellicle and green fluorescence throughout. A heavy white precipitate is formed.

FERMI'S SOLUTION.—There is prompt growth in Fermi's solution with a beautiful blue fluorescence sometimes becoming green but more often remaining blue though held for several weeks. A heavy wrinkled pellicle is formed which does not fall readily on shaking, but clouding usually remains weak. Thumm[7] states that all of the colors produced by fluorescent bacteria are due to the same pigment, the blue becoming green with the production of alkali by the organism.

The addition of dilute ammonia to blue cultures of *Bact. delphinii* in Fermi's solution turned them a vivid green. Blue and green cultures were tested for relative acidity with the following results:

Blue P_H 6.5 or +41.

Green P_H 6.6 or +34.

NITRATE REDUCTION.—Nitrate is not reduced. Tests were made with nitrate bouillon cultures 5 days old and 10 days old in which moderate clouding had taken place, using the starch-iodin-sulphuric acid test.

NITROGEN COMPOUNDS.—The ability of the organism to obtain its nitrogen from various nitrogen compounds was tested in 1 per cent water solutions of the following: peptone, asparagin, asparagin plus dextrose, ammonium citrate, ammonium tartrate and ammonium succinate. The results are shown in Table I.

[6] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. pl. 15. Washington, D. C. 1912.

[7] THUMM, K. BEITRÄGE ZUR BIOLOGIE DER FLUORESCIERENDEN BAKTERIEN. Arb. Bakt. Inst. Tech. Hochschule Karlsruhe 1: 291-377. 1897. Abstract in Smith, E. F. Bacteria in relation to plant diseases. 1: 238. 1905.

TABLE I.—*Sources of nitrogen*

	1 day old	4 days old	10 days old	20 days old
Peptone	Very weak clouding.	Good clouding, heavy pellicle.	Same as 4 days, some precipitate.	No further growth.
Asparagin + dextrose	....do	Weak clouding, delicate pellicle.	Moderate clouding, good pellicle.	Do.
Asparagin	....do	....do	Weak clouding, delicate pellicle.	Do.
Ammonium citrate	....do	Weak clouding, no pellicle.	Weak clouding, no pellicle.	Do.
Ammonium succinate	....do	....do	....do	Do.
Ammonium tartrate	No clouding	No growth	Very weak clouding	Do.

LITMUS SUGAR AGARS.—Litmus agar containing 1 per cent peptone and 1 per cent of saccharose, maltose, lactose, dextrose, galactose, levulose, l. arabinose, glycerin or mannit was used for stroke cultures. The litmus is reddened decidedly on the second day and is red throughout in 6 days in the presence of dextrose, levulose, l. arabinose or galactose. Saccharose causes no color change except sometimes a very feeble reddening after 2 to 3 weeks. Cultures with maltose, lactose, glycerin and mannit become decidedly blue, beginning on the second day.

When the same carbohydrates are added to beef infusion peptone litmus agar the organism produces a blue reaction with all of them.

FERMENTATION TUBES.—In fermentation tubes containing 1 per cent peptone water and 1 per cent of saccharose, lactose, maltose, dextrose, galactose, glycerin or mannit, there is no growth in the closed end and no gas. In dextrose and galactose the reaction is acid to litmus while the others are alkaline to litmus. They were tested after 1 week, 2 weeks, and 4 weeks. Saccharose gives a neutral or slightly acid reaction.

BLOOD SERUM.—Only a very moderate growth takes place on Loeffler's blood serum and there is no liquefaction.

TOLERATION OF SODIUM CHLORID.—The organism grows promptly and well in 1 per cent NaCl in +10 or +12 beef bouillon and fairly well in 2 per cent, weakly in 3 per cent, and some isolations make very weak growth in presence of 4 per cent NaCl.

OPTIMUM P_H IN BEEF BULLION.—The best growth was made in +16 (P_H 6.7) to +12 (P_H 7.1). There was gradual but finally very good clouding in +25 (P_H 5.6), but none in +30 (P_H 5.2). Moderate clouding occurs in 0 (P_H 8.2), weak clouding in −2 (P_H 8.4) and in −4 (P_H 8.6), but none in −9 (P_H 9.2).

TOLERATION OF ORGANIC ACIDS.—Tests made with citric, malic, and tartaric acids are summarized in Table II. It was found that the P_H value is the controlling element here, the organism being able to grow in the same P_H of all acids while varying in its tolerance of the different acids judged by their Fuller's scale values.

AMMONIA PRODUCTION.—There is strong ammonia production. Cultures in beef bouillon 2 to 17 days old were tested by suspending over them strips of filter paper wet with Nessler's solution. Browning of the paper began immediately and became intense on heating the cultures in a water bath.

HYDROGEN SULPHID PRODUCTION.—No hydrogen sulphid is given off from bouillon, Uschinsky's solution, Fermi's solution, or potato cultures. Tests were made by suspending filter paper wet with lead acetate water in the tubes. No discoloration of the paper occurred.

TABLE II.—*Toleration of organic acids*

Citric acid			Malic acid			Tartaric acid		
Fuller's scale	P_H	Growth	Fuller's scale	P_H	Growth	Fuller's scale	P_H	Growth
+17	.....	Prompt heavy clouding.	+17	.....	Prompt heavy clouding.	+17	.....	Prompt heavy clouding.
+28	5.7	Delayed heavy clouding.	+25	5.8	Delayed heavy clouding.	+24	5.7	Delayed heavy clouding.
+33	5.4	No growth.....	+33	5.2	No growth.....	+29	5.2	No growth.

INDOL PRODUCTION.—No indol is produced in 10 days' growth either in Dunham's solution or in Uschinsky's solution to which 2 per cent Difco peptone was added. Tests were made with sodium nitrite and sulphuric acid. Cultures of *Bacillus coli* tested at the same time gave a good pink reaction.

EFFECT OF DESSICATION.—The organism is rather resistant to drying. Small drops from 24-hour beef bouillon cultures were spread on sterile cover slips in sterile Petri dishes and kept in the dark. Most of the covers when dropped into tubes of bouillon after 9 days' drying caused clouding, many clouded the bouillon after 12 days' drying, and some were able to cloud the bouillon after 20 days' drying. Plates were poured from these last which gave pure cultures of the right organism.

THERMAL RELATIONS.—The organism grows at temperatures from 1° to 30° C. in beef bouillon +12, P_H 7.1, with an optimum growth at 25°. At 1° clouding began on the sixth day and gradually (within three weeks) became heavy and green fluorescent with a pellicle. At 30° growth was very weak, even after three weeks, and without either pellicle or green color. Fresh transfers to bouillon kept at 34°, 35°, and 37° for eight days, when removed to room temperatures (23° to 25°) failed to cloud although held for two weeks.

The thermal death point is 50° C. when test-tube cultures in beef bouillon (P_H 6.8 to 7.2) are exposed in a water bath for 10 minutes (tube diameter 16 mm.)

EFFECT OF FREEZING.—Transfers were made to beef bouillon (+12, P_H 6.7) from 24-hour-old bouillon cultures. These were allowed to stand 10 minutes, shaking at intervals; then beef agar plates were poured. The freshly transferred cultures were then frozen solid in a salt and ice mixture, kept frozen for 15 minutes and thawed in cool water. Plates were then again poured as before. From 40 per cent to 60 per cent of the bacteria were killed by this treatment.

EFFECT OF SUNLIGHT.—Tests were made in Washington, D. C., in August. There were no clouds but the sky was not brilliantly clear. Plates poured from 24-hour bouillon were exposed bottom side up on ice to the direct rays of the sun. One-half of each plate was protected from the sun by several folds of black paper. Exposures were made for 5, 10, 20, 30, 45, and 60 minutes. A colony count gave the following results: One-third were killed by a 5-minute exposure, one-half by 10-minute, three-fourths by 20-minute and all by 45-minute and 60-minute exposures.

LONGEVITY.—The organism lives at room temperatures for six months in beef bouillon and in milk, or until the culture is actually almost completely evaporated.

GROUP NUMBER.—The group number is 211.2322123 according to the descriptive chart of the Society of American Bacteriologists.

TECHNICAL DESCRIPTION

Bacterium delphinii (EFS).

A short nonsporiferous rod with rounded ends; usually single or in pairs, sometimes in short chains; flagella 1 to 6 bipolar: capsules, aërobic, white, but producing a blue-green fluorescence in cultures; diastasic action weak; liquefies gelatin; does not reduce nitrates; clears milk without coagulation; blues, then reduces litmus milk; produces ammonia but no hydrogen sulphid or indol; forms acid from dextrose, galactose, levulose, and with more difficulty from saccharose, but not from lactose, maltose, glycerin or mannit; does not produce gas from any of these carbon compounds; grows well in Uschinsky's, Fermi's and Cohn's solutions; optimum temperature 25° C., maximum 30°, minimum 1°, or less; thermal death point 50°; Gram-negative; not acid-fast; stains readily with carbol fuchsin and gentian violet; is pathogenic to delphiniums, producing dead black spots on the leaves, stems and flower buds.

CONTROL METHODS

It has been found impractical to do any control work in Washington. The delphinium plant does not reach its best development here, preferring the cooler northern States. The organism also is very sensitive to high temperatures. Moreover, isolations kept on culture media over winter have lost much of their virulence and by the time fresh isolations can be obtained from northern material the weather here has become unfavorable to both host and parasite. Control studies should therefore be made in a region where the disease prevails.

A few suggestions may be made, however. It seems probable that the organism winters-over in the soil since it is rather resistant to cold and drying, and since infection generally appears first on the lower leaves, suggesting the spattering of infected soil by rain. On this theory all diseased material should be gathered and burned. Drenching the soil surrounding the plants with alkaline Bordeaux mixture before growth begins in the spring and later spraying both the soil and the lower surface of early leaves with Bordeaux mixture should prevent infection from this source.

SUMMARY

The black spot of delphinium is a bacterial disease widespread in the northern part of the United States and is very destructive to choice hybrid varieties.

The bacteria gain entrance to the plant through the water pores and stomata, causing irregular tarry black spots on the leaves and sometimes on stems and flower buds.

The causal bacterium has been isolated and successful infections obtained by spraying with water suspensions of young subcultures from single colonies.

For control of the disease it is suggested that all diseased material be collected and burned and that the surrounding soil and early leaves be sprayed with Bordeaux mixture.

Since the flagella have been demonstrated to be polar, the name of the organism becomes *Bacterium delphinii* (EFS).

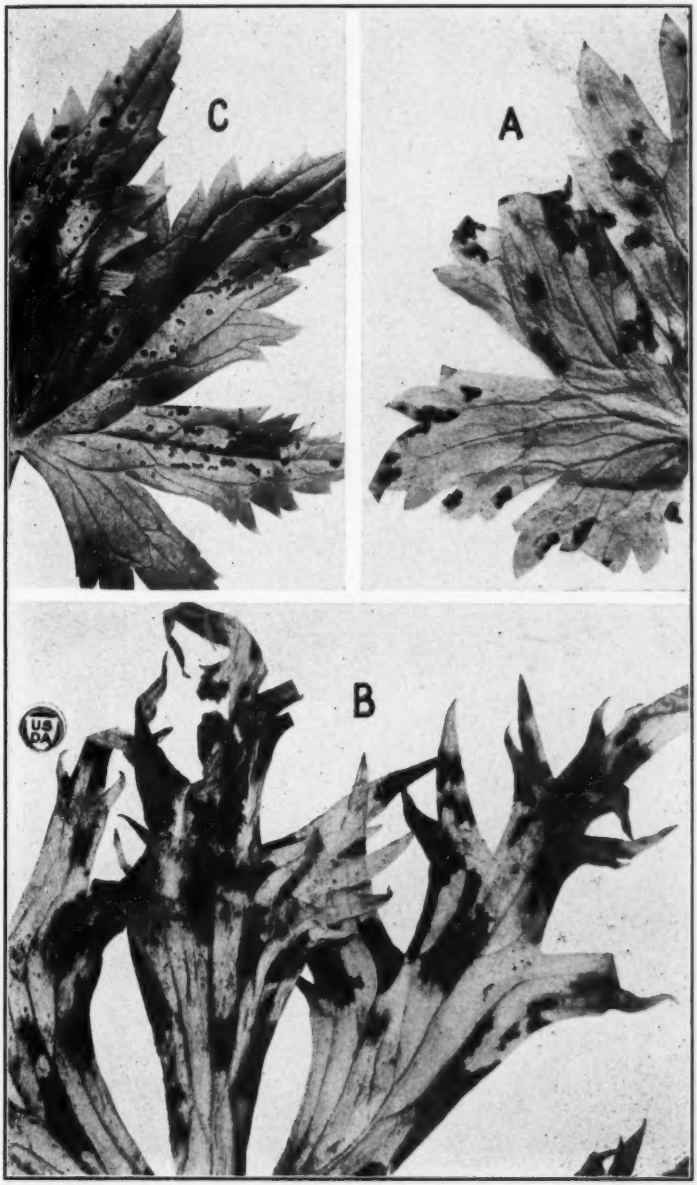
PLATE 1[a]

A.—Delphinium leaf from Maine, 1923. Small spots.

B.—Delphinium leaf from Castine, Me., 1922. Large spots caused by *Bact. delphinii*. Reduced slightly.

C.—Spots on delphinium leaf produced by spraying with water suspension of a subculture of a single colony freshly isolated from a leaf from Maine, in July 1923, 32 days after inoculation.

[a] All photographs were made by James F. Brewer.


C
A
B
USDA

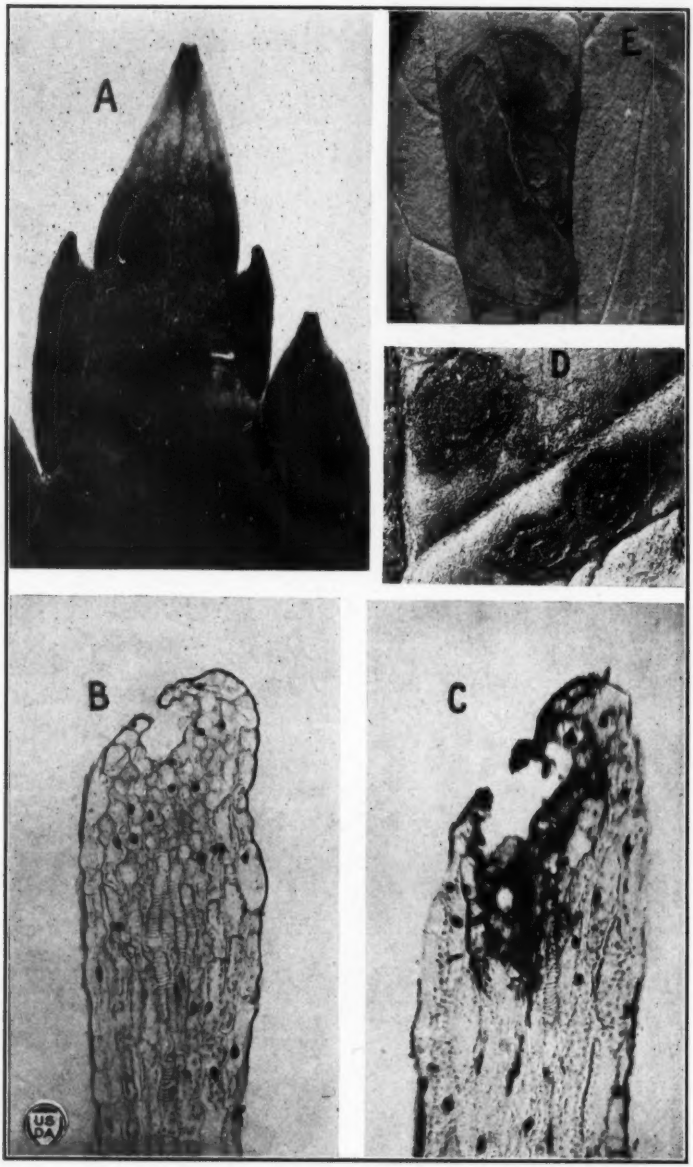

A
E
D
B
C
USDA

PLATE 2

A.—Tip of a delphinium leaf showing infected (blackened) water pores. Time, 20 days after inoculation in the hothouse with the organism from Oregon. × 10.

B.—Microtome section of a normal water pore. The black spots are nuclei.

C.—Microtome section of an infected water pore.

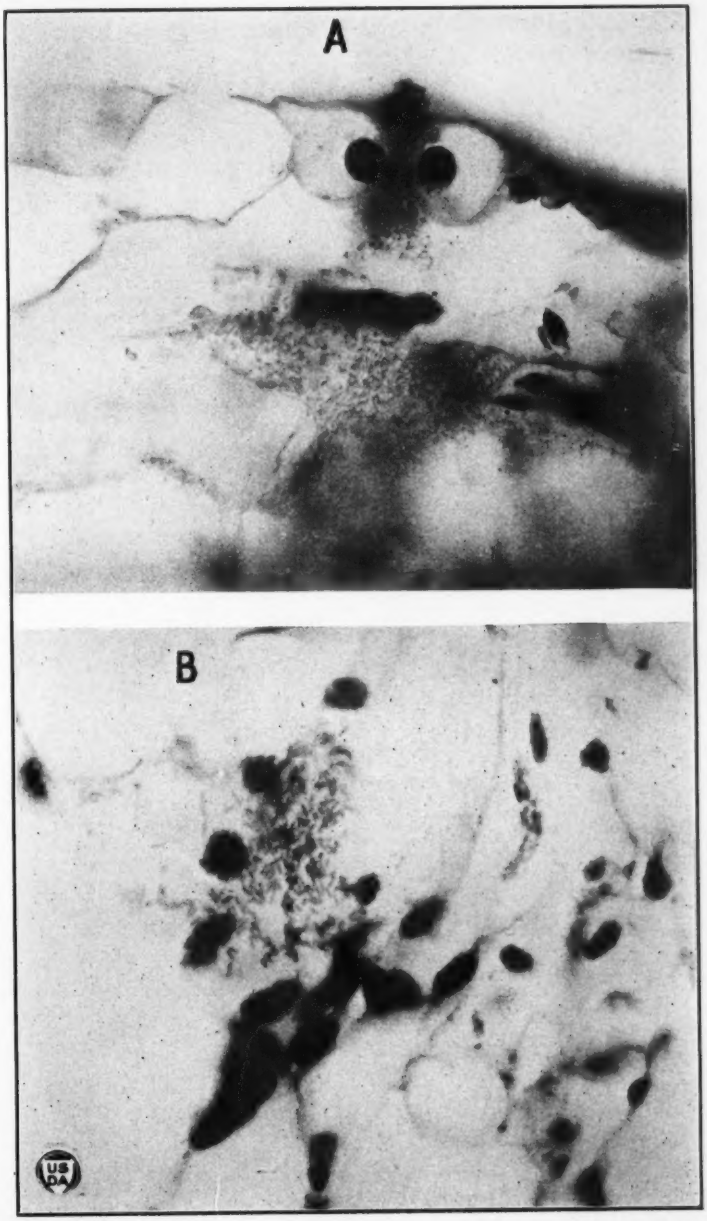
D.—Small leaf spots showing zoning. Photographed from a fresh leaf received from Connecticut. × 4.

E.—Large leaf spot showing zoning. Photographed from a pressed leaf from Castine, Me. × 2½.

PLATE 3

A.—Stomatal infection. Microtome section of a leaf spot 48 hours after inoculation by spraying. Bacteria lie on the walls of the cells nearest the stoma, and in and under the stoma. × 1400.

B.—Bacteria in the tissues. The large oval bodies are masses of bacteria. × 1400.


A
B

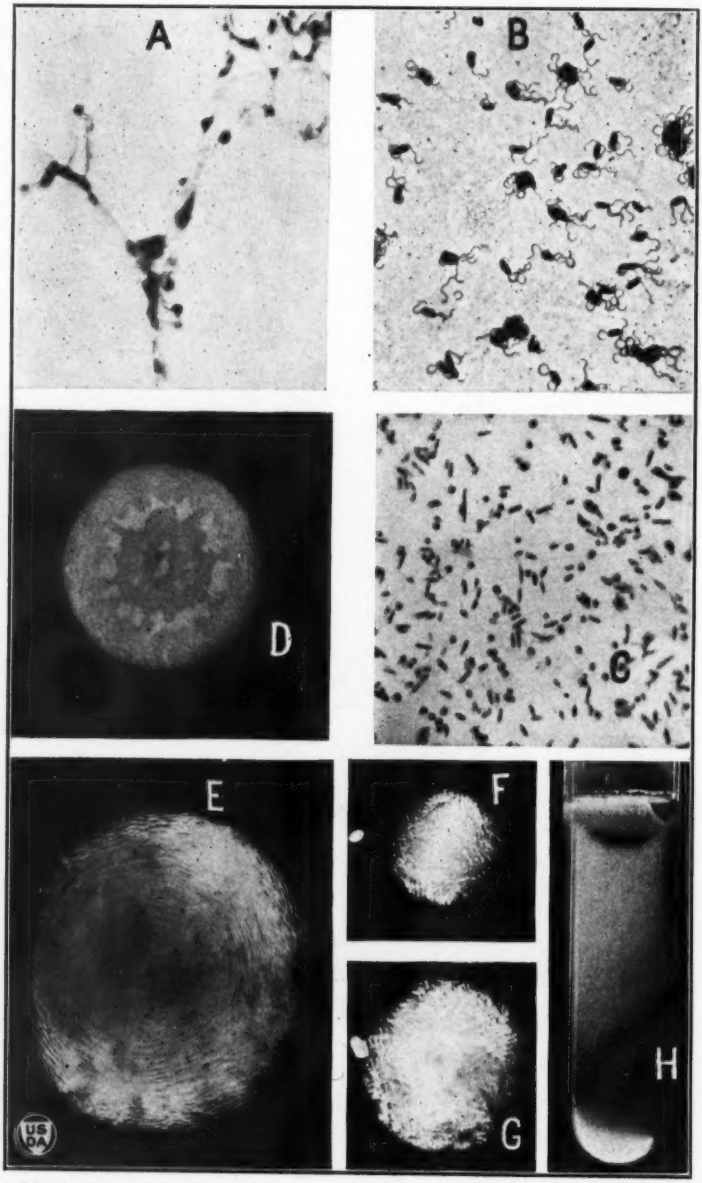

A
B
D
C
E
F
G
H
U.S. D.A.

PLATE 4

A.—Hemispherical extrusions on cell walls in the vicinity of the invading bacteria. × 500.

B.—*Bacterium delphinii* showing flagella. Stained by Casares-Gil's method. × 940.

C.—Rods and spherical bodies from the sediment in the pellicle of bouillon cultures.

D.—Agar plate, colony 4 days old showing ring of opaque areas. × 10.

E.—Agar plate, colony 9 days old, by oblique transmitted light. × 10.

F.—Agar plate, colony 2 days old, by oblique transmitted light. × 10.

G.—Agar plate, colony 3 days old, by oblique transmitted light. × 10.

H.—Culture in beef bouillon 4 days old, showing pellicle weighted down in center by a mass of sediment.

FURTHER OBSERVATIONS ON THE MOLTS OF THE OX BOTS HYPODERMA BOVIS DE GEER AND H. LINEATUM VILLERS[1]

By E. W. LAAKE

Entomologist, Investigations of Insects Affecting the Health of Animals, Bureau of Entomology, United States Department of Agriculture

INTRODUCTION

In a paper [2] published by the writer the conclusion was reached that there are five stages or four molts in the larvæ of *Hypoderma lineatum* Villers and probably the same number in *H. bovis* De Geer. These conclusions have been generally accepted as presented by the writer but they have been questioned by some entomologists who hold to the older theory of the occurrence of only three stages or two molts in the case of Oestridae as well as other Diptera. The purpose of the present paper, therefore, is to present further facts substantiating the claim that there are five larval stages in these two species of Hypoderma.

The writer's paper referred to above, setting forth the larval characters for each stage, was brief, and merely the general differences of the cephalopharyngeal skeleton, mouth parts, and spinous armature of each species were given.

The confusion in the larval stages lies in the earlier ones, that is, first, second, and third. The first stage as hatched is well known, as are also the last two stages in the back of the host. Some maintain that the larvæ have no molt from the time they leave the egg and enter the skin of the host until they reach the subdermal tissues of the back approximately eight months later, during this time having increased in length from less than 1 mm. to from 13 to 16 mm.

COMPARISON OF CHARACTERS OF FIRST THREE LARVAL STAGES IN HYPODERMA LINEATUM

For the purpose of comparing some of the more outstanding characters of each of the first three larval stages as described by the writer, three larvæ of each stage taken at random out of a large series of cleared and mounted specimens of *Hypoderma lineatum* were subjected to a microscopic examination, the characters of each instar carefully noted, and measurements made and averaged for the three specimens of each instar. These averages are presented in Table I. The characters noted in the table constitute measurements of the cephalopharyngeal skeleton with mouth hooks, the number of spines in the group immediately below the mouth, the spines on the ventral side of the eighth segment and those on the anal segment, including the flattened or triangular spines on the border of the posterior spiracles. The eighth segment was chosen for special study because the diameter of the larva is near its greatest at this point and the counts and measurements of spines can be made with greater accuracy and ease than on the smaller segments.

[1] Received for publication March 21, 1924.

[2] LAAKE, E. W.—DISTINGUISHING CHARACTERS OF THE LARVAL STAGES OF THE OXWARBLES HYPODERMA BOVIS AND HYPODERMA LINEATUM, WITH DESCRIPTION OF A NEW LARVAL STAGE. Jour. Agr. Research 21: 439–457, illus. 1921.

TABLE I.—*Dimensions in microns of the cephalopharyngeal skeleton and mouth hooks and the number and dimensions of part of the spinous armature of the first, second, and third larval instars of Hypoderma lineatum*

Instar	Total length	Expanse of mouth hook from tip to tip	Length of cephalopharyngeal skeleton and mouth hooks	Number of spines in group below mouth	Diameter of eighth segment	Spines on ventral side of eighth segment: First row: Diameter at base: Minimum	Diameter at base: Maximum	Length: Minimum	Length: Maximum	Distance apart: Minimum	Distance apart: Minimum	Number in row
1	419	14.1	115.6	36	114	2.6	3.4	5.4	6.3	1.5	3.3	23.0
2	2,312	15.3	117.1	55	731	2.7	3.5	4.5	9.8	17.0	25.6	30.6
3	13,422	16.6	125.5	57	3,462							0.0

Instar	Spines on ventral side of eighth segment: Second row: Diameter at base: Minimum	Diameter at base: Maximum	Length: Minimum	Length: Maximum	Distance apart: Minimum	Distance apart: Maximum	Number in row	Third row: Diameter at base: Minimum	Diameter at base: Maximum	Length: Minimum	Length: Maximum	Distance apart: Minimum	Distance apart: Maximum	Number in row
1	2.0	2.9	4.1	5.5	1.6	3.9	24.0	1.8	2.5	2.9	3.8	3.0	5.0	22.3
2	1.9	2.8	4.2	8.2	12.8	31.6	29.6	1.6	2.4	4.5	6.1	10.9	36.3	30.6
3							0.0							0.0

Instar	Spines on ventral side of eighth segment: Fourth, fifth, sixth and seventh rows: Diameter at base: Minimum	Diameter at base: Maximum	Length: Minimum	Length: Maximum	Distance apart: Minimum	Distance apart: Maximum	Number in row	Anal segment: Spines: Diameter at base: Minimum	Diameter at base: Maximum	Length: Minimum	Length: Maximum	Length of triangular spines on border of posterior spiracles	Diameter of posterior spiracles
1	1.4	2.9	2.1	4.7	2.3	6.6	21–23	2.4	3.3	4.3	7.3	12.1	5.9
2	1.4	2.3	3.7	6.1	11.6	37.6	29–31	2.7	7.6	4.2	10.5	10.3	9.4
3							0	6.5	22.5	5.7	17.4	5.6	14.1

The figures in the table reveal marked differences, particularly in the number and the dimensions of the spines of the stages in question. The cephalopharyngeal skeleton and mouth hooks increase in size, as shown by the figures in the table, and the posterior part of the skeleton changes its shape from one stage to the next, spreading out with each molt. Although these differences are not great, there is shown, nevertheless, constant increase in the size of these highly chitinized organs.

If the spines in the group below the mouth are next examined a wide difference in number is found, especially between those of the first and second stages. Since the figures in the table represent averages for each stage, this difference could not be accounted for by individual variation. Among the spines on the ventral side of the eighth segment just as marked a difference in dimensions and number is found. With only one exception, that of the first row, the maximum diameter of the spines at the base is larger in the first than in the second stage. Although the diameter of these spines has decreased slightly from the first to the second stage, the length, without exception, has nearly doubled and the number of spines has likewise increased from 23 to 30.6 for the first row and similarly for all the other rows. Finally, when the spinous armature of the terminal segment is considered, the most striking differences are found. The spines

of the first-stage larva are straight or only slightly curved, with a somewhat blunt point and without a large and heavily chitinized base. The maximum length of the spines is more than twice the maximum diameter at the base. The triangular spines on the border of the posterior spiracles are well pointed, more heavily chitinized, and longer than the other spines on the terminal segment.

In the second stage the spines are very sharply pointed, strongly curved, and only slightly longer than the large, circular, elevated base. The shape of these spines, in every respect, is distinctly different from that found in spines on the first stage. The triangular spines on the border of the spiracles are equal in length or slightly shorter than the longest spines on the terminal segment. They are without a distinct point and shorter than in the first stage. The diameter of the spiracles has increased considerably compared with that of the first stage. In the third stage the spines on the terminal segment again show a striking difference from those of the second stage and the greatly increased dimensions are in a reverse order. The diameter of the enlarged base, nearly three times that of the second stage, is now greater than the length of the spine, which itself has attained a length almost twice that of the second stage. The posterior spiracles have increased their diameter considerably and the triangular spines on the border of the spiracles have decreased in size to almost half that of the preceding stage and are now very blunt, or sometimes slightly knobbed at the point.

Gedoelst,[3] who holds that there are only three stages in the larva of Hypoderma, states that when growth takes place within the duration of the same stage it comprises all the soft parts of the organism, but that it does not affect the highly chitinized organs, such as the stigmata, cephalopharyngeal skeleton, and spines, which do not acquire any greater dimensions, except on the occasion of molts. He attributes the greater distance between the spines of the larger (second-stage) larvæ to the growth of the soft parts of the organism, and explains the vastly greater dimensions of the spines on the terminal segment as a continuation of the processes of chitinization.

The greater distance between the spines of the second-stage larvæ could easily be the result of growth of the soft parts of the organism but in such case the number and size of the spines should remain constant. This, however, is not the case. Certainly the increase in the number of spines could not be explained as an extension of the processes of chitinization. By this theory it would seem even more difficult to explain the absence of spines on the body segments of the third-stage larvæ. Koch,[4] who also holds that there are only three larval stages in Hypoderma, advances the theory that the spines on the body segments are gradually worn down as the larvæ migrate through the tissues. This would contradict Gedoelst's theory of the continuation of the processes of chitinization as the cause of the enlargement of the spines. Koch's assumption was not correct, however, at least not in so far as the first and second-instar larvæ are concerned, as the spines of the second-stage larvæ, although actually slightly smaller in diameter, with the exception mentioned before, are much longer than in the first stage.

It is not likely that the clearing process in preparing the specimens for slide mounts could be responsible for the disappearance of the spines on all the body segments in the third-stage larvæ and not affect the small spines in the group below the mouth or any of the spines in second-stage larvæ.

Further evidence that there is a molt between the smaller spiny larvæ and the larger spineless larvæ is the fact that occasionally a larva is found that possesses one or sometimes several small groups of highly chitinized spines on

[3] GEDOELST, L.—LE TRIMORPHISME LARVAIRE DES OESTRIDÉS. Compt. Rend. Soc. Biol. [Paris] 90: 501–504. 1922.

[4] KOCH, T. P.—OM OKSEBREMSEN HYPODERMA BOVIS. Maanedskr. Dyrlaeger 15: 129–139. 1903

some one of the body segments. These spines, although similar in shape and size to those in rows on the earlier stages, are not placed in regular order as they appear in either the first or second stage. The occurrence of a small group of irregularly arranged spines on the otherwise spineless body segments of the third stage can be explained as an occasional individual variation, and clearly indicates their presence as the result of a molt, as spines so arranged are never present in the first or second instars.

Although the characters as set forth above should be sufficient to show clearly that there are at least three distinct stages of *Hypoderma lineatum* in the course of the migration of the larvæ from the point of entrance to the place of encystment on the back, where two subsequent larval molts take place, it has been the author's good fortune to obtain two larvæ of *H. lineatum* during the process of molting from the second to the third stage, thereby definitely establishing this fact for one of these molts, at least. These larvæ, both approximately 13 mm. in length, were taken, one from the gullet and one from the diaphragm of two different animals slaughtered in a packing house at Dallas, Tex.

The specimen taken from the gullet, although almost completely free from its cast-off second-stage exuvium, was still possessed of part of it. The newly molted specimen, a third-stage larva, is free from spines on the body segments while the remaining part of the exuvium extending back nearly as far as the third segment of the larva, and plainly showing the torn borders, is covered with the usual second-stage armature. In the case of the larva taken from the diaphragm, the entire and unbroken second-stage exuvium was present and was easily removed from the smooth larva. This newly molted third-stage specimen, spineless on all the body segments, together with the cast-off second-stage exuvium possessed of the entire spinous armature of that stage, was cleared, mounted, and preserved.

The rarity of finding a larva in the process of molting is no doubt due to the very delicate structure of the exuvium and the extreme difficulty in removing the larva from the connective tissue of the host without losing the skin. In the case of the first molt, which probably takes place soon after the larva gains entrance to the host, when it is extremely small, it is almost hopeless to obtain a specimen at the instant of molting from the first to the second stage. In fact, no first-instar larvæ have ever been found after having left the point of entrance.

THE SECOND-STAGE LARVA OF HYPODERMA BOVIS

The second-stage larva of *Hypoderma bovis* was unknown in 1921, at the time the writer's preceding paper was published, but its existence was considered probable and deemed necessary to fill the gap between the first and third stages which are so similar to those stages in *H. lineatum*. Since then Phibbs [5] of Ireland has collected and described this stage. At about the same time Wells and Brundrett, of the Bureau of Entomology, who conducted investigations in the State of New York, made a number of collections of second-stage larvæ of *H. bovis*, all of which, with the exception of a single specimen from the gullet, were taken from the neural canal of slaughtered cattle.

The characters of the second-stage larva of *Hypoderma bovis* are remarkably similar to those of *H. lineatum* in the same stage, except, of course, the difference, as pointed out before, in the mouth hooks and in the anterior end of the cephalopharyngeal skeleton. The arrangement of the spinous armature of the head segments, the transverse rows of spines on the body segments, with the heavier spines in the anterior rows of each segment, and the spines, with broad bases, on the anal segment are all almost identical in appearance with those of *H. lineatum* in the same stage.

[5] PHIBBS, G.—THE LARVAL MOUTH-HOOKS OF HYPODERMA. Irish Nat. 31: 25-30, illus. 1922.

FLAVOBACTERIUM SUAVEOLENS, A NEW SPECIES OF AROMATIC BACILLUS ISOLATED FROM DAIRY WASTES[1]

By LULU SOPPELAND

Iowa State College of Agriculture and Mechanic Arts and Iowa Engineering Experiment Station

INTRODUCTION

In a large group of organisms isolated from dairy wastes were found several which produced an aromatic odor from all of the mediums containing protein. The odor is sweet-scented, very pleasing during the first two days' incubation at 22° C. but in four to six days it changes to a decided cheesy odor which persists for two months. At 37° the cheesy odor develops in two days. During the three years these organisms have been under observation, this property of aroma production has always been manifested in subcultures, apparently undiminished in intensity.

As the aroma-producing property seemed to be associated with the nitrogenous organic matter in the medium, it was anticipated that the organism was an active proteolyzer. Inoculated into a 1½ per cent gelatin with an initial P_H of 7.3 and held at 22° C. for six days, the amino and ammoniacal nitrogen increased from 50 to 950 p. p. m. Inoculated into skim milk and kept under similar conditions, the amino and ammoniacal nitrogen increased from 30 to 300 p. p. m.

MORPHOLOGY

Smears from plain agar (12 hours—7 days) are always Gram-negative. Milk powder agar cultures, 12 to 16 hours old, are Gram-positive and become Gram-negative on further incubation; spores were not observed when tested by spore stains (24-hour to 7-day old agar culture) or by exposure to temperatures of 60° to 80° C.; growth was not obtained after heating either a young or old culture at 60° for 10 minutes or at 80° for 5 minutes; size varies from 1.0μ to 1.2μ by 0.6μ to 0.8μ; rods have rounded ends; actively motile, position of flagella peritrichous.

CULTURAL CHARACTERISTICS

Records are for 22° C. for 48 hours unless otherwise stated. The P_H of media employed ranged from 6.5 to 7.5

NUTRIENT-AGAR SLANT.—Growth is moderate, filiform, flat, glistening, opaque, of butyrous consistency and produces an aromatic odor; pigment is yellow—510.033 shade 2[1]; pigment is insoluble in water, methyl, ethyl, and isoamyl alcohols, benzene, carbon tetrachlorid, and xyol; readily soluble in acetone and slowly soluble in ether and chloroform; in 6 days growth is oleaginous.

NUTRIENT-AGAR PLATES.—Colonies small, growth similar to slant; confluence marked in seven days.

MILK POWDER AGAR SLANT.—Similar to plain agar but growth more abundant and color deeper—420.042 shade 3[1]; proteolysis distinct.

NUTRIENT BROTH.—Moderate uniform clouding, scanty sediment; no surface growth; an aromatic odor which changes to cheesy in four days.

GELATIN STAB.—A stratiform liquefaction is visible in one-half day; gelatin completely liquefied in six days; medium brownish and white precipitate settles at bottom by sixth day.

POTATO SLANT.—Abundant growth, color more intense than on nutrient or milk powder agar—330.051 shade 3[1].

[1] Received for publication Jan. 26, 1924.

PHYSIOLOGICAL REACTIONS

The carbohydrates are weakly attacked. In seven days a slight acidity but no gas can be detected in dextrose, sucrose and glycerin broths[2]; neither acid nor gas in lactose or starch; in a 0.4 per cent caseinate solution plus 1 per cent lactose, the P_H changed from 7.0 to 6.9 in three to seven days at 22° C.; no diastatic action was evident on starch agar.

MILK.—The organism proteolyzes milk rapidly; the reaction remains unchanged or becomes slightly alkaline; no curd can be detected even by heating but on long incubation a powdery amorphous precipitate develops; litmus reduces in seven days. In whole milk the cream layer is unaffected; tubes of whole milk incubated at 37° C. or at 22° for two months did not have the cream layer visibly altered; the milk beneath the cream layer is digested nearly as rapidly as in skim-milk tubes; the odor remains cheesy, but is not considered putrid.

NO_3 BROTH.—Nitrates are not reduced in 10 days at 22° C.

INDOL PRODUCTION.[3]—Indol present in seven days at 22° C.

H S PRODUCTION.[4]—H_2S is produced in three to seven days at 22° C.

RELATION TO OXYGEN.—Grows best aerobically.

Omelianski (*2*)[5] in a recent article quite thoroughly reviews the literature on aroma-producing microorganisms. Several species have been described which bear close resemblance to the organisms under consideration. Among them are *Bacillus praepollens* Maaszen (*1*) and *Flavobacterium aromaticum* Pammel (*3*). These, however, differ in several characters. *Bacillus praepollens* of Maaszen is nonmotile, does not produce indol and produces black granules in broth and in gelatin. Dr. Pammel's *F. aromaticum* is indol-negative and produces gas from dextrose and sucrose.

A thorough search of the literature has not revealed a previous description which adequately fits this organism. In the absence of a previous description, the Latin word suaveolens, meaning sweet smelling or fragrant, is suggested as the species name. According to Bergey's "Manual of Determinative Bacteriology" (*4*) it belongs in the genus *Flavobacterium*—hence the name *Flavobacterium suaveolens*.

LITERATURE CITED

(1) MAASZEN, A.
1899. FRUCHTÄTHERBILDENDE BAKTERIEN. Arb. K. Gsndhtsamt 15: 500–513, illus.

(2) OMELIANSKI, V. L.
1923. AROMA-PRODUCING MICROÖRGANISMS. Jour. Bact. 8: 393–419.

(3) PAMMEL, L. H., and PAMMEL, E.
1896. A CONTRIBUTION ON THE GASES PRODUCED BY CERTAIN BACTERIA. Centbl. Bakt. (II), 2: 633–650, illus.

(4) SOCIETY OF AMERICAN BACTERIOLOGISTS.
1923. BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY. 442 p. Baltimore.

[2] Medium employed:
1 per cent Andrade's indicator
1 per cent peptone
0.5 per cent NaCl
0.2 per cent K_2HPO_4
1 per cent carbohydrate.

[3] This was tested according to Malone and Gore's cotton plug test.

[4] The method of the Society of American Bacteriologists, 1920, was used.

[5] Reference is made by number (italic) to "Literature cited," p. 276.

SOME RELATIONS BETWEEN THE GROWTH AND COMPOSITION OF YOUNG ORANGE TREES AND THE CONCENTRATION OF THE NUTRIENT SOLUTION EMPLOYED [1]

By H. S. REED, *Professor of Plant Physiology*, and A. R. C. HAAS, *Assistant Professor of Plant Physiology, College of Agriculture, University of California*

INTRODUCTION

The present paper is devoted to a discussion of the effects of different concentrations of the nutrient medium upon young Valencia orange trees (*Citrus sinensis*) and seedlings of the African sour orange (*Citrus aurantium*).

THE RELATIVE CONSTANCY OF THE CONCENTRATION AND REACTION OF LEAF SAP

The data on this point were obtained from young Valencia orange trees in cultures like those which have been previously described by the writers (*3*) [2]. The nutrient solutions applied were either Hoagland's solution (*3*) or modifications thereof. None were strongly toxic although growth was greatly retarded in certain series. Their osmotic concentrations as determined from the freezing-point depression ranged from 0.531 to 1.568 atmospheres, and their P_H values ranged from 5.2 to 7.5. The plan of the experiments made it impracticable to determine the reaction of the nutrient solution after it was added to the sand cultures, but the reaction of the first 100 cc. of percolate collected after adding distilled water to the surface of the cultures was determined. The first drops collected had the highest P_H values. All percolates had higher P_H values than the nutrient solutions which had been applied. In the case of solutions which contained 1,000 parts per million of sodium bicarbonate (series 30–35 and 36–41) the P_H of the percolates was approximately 8.3. The concentration of OH ions in the percolate from soil cultures which had previously received sodium chlorid solution was equally high. The replacement phenomena involved in such cases have been discussed by Cummins and Kelley (*1*).

The data (Table I) show that the P_H and concentration of the leaf sap from trees in the various cultures were remarkably uniform. The small differences found seem to bear no relation to the P_H or to the concentration of the nutrient solution nor to the P_H of the percolates. It appears that the absorption and metabolism of the orange trees produce a fairly constant reaction and concentration of the leaf sap. These results are not necessarily contradictory to those obtained by Haas (*2*) with the sap of *Melilotus alba*, where it was found that a gradient may exist in the plant. It is essential, however, that other plants be examined because any hypothesis based upon differences of acidity of the plant sap and the external medium must account not only for the accumulation of inorganic elements within the roots, but also for their movement to other portions of the plant in which different acidities may prevail. Moreover, the conditions of solubility in the root system may not be the same as those in other parts of the plant.

[1] Received for publication Nov. 26, 1923, Paper No. 116, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

[2] Reference is made by number (italic) to "Literature cited," p. 284.

THE EFFECT OF CONCENTRATION UPON THE GROWTH AND COMPOSITION OF ORANGE SEEDLINGS

A series of water cultures was conducted in a glass house for a period of 122 days to study the effects of six concentrations of nutrient solution upon seedlings. The concentration of the nutrient solutions ranged from 364 to 3635 parts per million. The solution used as a standard had the following composition expressed in parts per million:

Na	K	Ca	Mg	Mn	Fe	Cl	NO_3	SO_4	PO_4	Total
7	185	158	54	Trace.	Trace.	10	718	218	105	1,454

If we represent the total concentration of the standard as C, we may designate the series employed as .25 C, .50 C, 1.0 C, 1.5 C, 2.0 C, and 2.5 C. Eighteen 1-liter glass jars each containing three African sour orange seedlings (*Citrus aurantium*) were used for each of the six concentrations. Each week the solutions in the jars were changed and the water loss measured. At the end of the 122-day period the 54 seedlings used in each set of cultures were removed, weighed, and dried at a temperature between 60° and 70° C. The tops had attained a height of about 15 cm. The leaves, stems, and roots were kept separate for further determinations.

TABLE I.—*Relations between the concentration and reaction of nutrient solution and of the leaf sap of trees to which the nutrient solution was applied*

Series	Nutrient solution		Percolate	Leaf sap	
	Osmotic pressure	P_H	P_H of first portion of percolate	P_H	Osmotic pressure
SAND CULTURES	*Atm.*				*Atm.*
1–5	0.728	5.2	5.9–7.2	6.0	20.8
6–11	1.459	5.2	6.6–8.0	5.88	22.59
12–17	1.568	5.2	5.4–7.8	5.88	21.26
18–23	1.001	5.2	6.4–8.0	5.85	23.10
24–29	1.218	5.2	5.8–7.35	5.87	20.45
30–35	1.278	7.55	8.3+c	5.95	21.47
36–41	1.447	7.45	8.3+c	5.80	21.17
42–46	0.708	5.2	7.0–7.4	5.95	22.53
47–51	0.531	5.2	6.0–7.2	5.77	21.89
SOIL CULTURES					
84–88	----------	(a)	----------	5.80	21.03
89–92	----------	(b)	(c)	5.82	20.32

a Distilled water only was added.
b Distilled water containing 1,500 parts per million NaCl was added.
c Sodium carbonate present.

The data on the weight of the plants and of their ash are given in Table II, and show that the best growth of seedlings was made in 1.5 C, a solution which had an initial concentration of 2,181 parts per million. The plants grown in .25 C and .50 C were inferior to those in the other concentrations. The differences in both green and dry weight of tops were greater than for the corresponding weights of roots; in fact, the latter were rather uniform. By way of comparison, it may be stated that 54 seedlings of the size of those used to start these cultures had a green weight of 21.4 gm. and a dry weight of 3.52 gm.

The data on transpiration per unit of dry weight of tops show considerably more variation than when computed per unit of dry weight of the entire plant. The

ratio of water transpired to unit of dry weight was least in case of the largest plants (as is usually the case), and greatest in the case of the smallest plants.

Table III shows data obtained from the analysis of the leaves and stems of the seedlings just described. The leaves showed an increasing percentage of ash, with increasing concentration of culture solution. The percentage of calcium in the ash of the leaves showed a steady decline in the lowest four concentrations, while the percentages of potassium showed a rise in the higher concentrations. On the other hand, the percentage of sulphate and phosphate was lowest in the cultures receiving the three highest concentrations.

The ash of the stems increased with increasing concentrations of culture solution, but the percentage of ions in the ash showed no pronounced changes corresponding with increasing concentrations of culture solution. The last column in Table III shows the composition of entire seedlings from the same source and of the same age as those used in installing the cultures. Attention should be paid to their content of sodium, potassium, and phosphoric acid. Computation of the actual amounts of sodium and phosphoric acid in the dry matter of the seedlings shows that the stems have lost rather than gained in these ions during the course of growth in 122 days.

TABLE II.—*African sour orange seedlings in various concentrations of nutrient solution. (Each lot consisted of 54 seedlings having an initial green weight of 21.4 gm. and dry weight of 3.52 gm.)*

		CONCENTRATION OF NUTRIENT SOLUTION					
		.25 C	.50 C	1.0 C	1.5 C	2.0 C	2.5 C
		Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
Green weight	Tops	37	44	66	92	84	78
	Roots	45	39	51	58	52	55
	Entire plants	82	83	117	150	136	133
Dry weight	Leaves	6.84	8.76	13.39	19.09	16.80	14.07
	Stems	4.45	4.03	5.80	8.10	6.36	6.50
	Roots	8.91	7.79	10.47	11.87	10.60	11.00
	Tops	11.29	12.79	19.19	27.19	23.16	20.57
	Entire plants	20.20	20.58	29.66	39.06	33.76	31.57
Weight of ash	Tops	0.869	1.072	1.759	2.629	2.656	2.552
	Roots	0.756	0.696	1.055	1.224	1.228	1.216
	Entire plants	1.625	1.768	2.814	3.853	3.884	3.769
Water transpired per gram of dry weight.	Tops	893	826	673	564	603	633
	Entire plant	499	514	435	393	414	412

TABLE III.—*Composition of the ash of the leaves and stems of orange seedlings from solutions of various concentrations*

Concentration of nutrient solutions	Leaves						Stem						Entire seedlings from germinating box
	0.25 C	0.50 C	1.0 C	1.5 C	2.0 C	2.5 C	0.25 C	0.50 C	1.0 C	1.5 C	2.0 C	2.5 C	
Percentage of ash in the dry matter	8.50	8.68	9.59	10.24	12.11	13.16	6.49	7.73	8.19	8.31	9.77	10.79	5.58
Percentage of water soluble inorganic matter	41.03	46.66	51.39	51.99	60.18	57.68	59.19	61.11	62.09	66.67	67.49	69.07	.....
Constituents as a percentage of the ash:													
Na	3.05	3.88	5.10	6.31	7.70	6.60	4.54	6.17	5.07	6.11	6.68	.....	13.29
K	15.39	20.77	23.35	23.66	28.02	26.87	25.94	27.75	27.93	30.11	29.07	.....	27.73
Ca	17.62	14.55	11.13	7.46	7.77	8.85	13.90	13.52	13.01	11.61	9.43	10.96	6.32
Mg	3.12	2.25	3.90	.....	.....	.....	3.40	3.92	2.88	3.08	4.50	3.91	3.51
Cl	.46	.71	.78	.53	1.10	.37	1.91	2.31	2.74	1.90	2.90	1.31	1.07
SO_4	7.66	6.31	6.24	5.30	4.97	5.14	6.31	5.88	6.23	4.74	5.10	5.41	5.45
PO_4	8.36	7.75	8.41	5.66	5.31	5.66	13.59	10.40	10.69	11.16	9.20	9.21	26.97

THE EFFECT OF CONCENTRATION UPON THE GROWTH AND COMPOSITION OF YOUNG VALENCIA ORANGE TREES IN SAND CULTURES

In experiments on this question we employed 1-year-old orange trees growing in large galvanized iron cans containing silica sand. The cans were provided with means for adding nutrient solutions and collecting drainage water according to methods previously described (*3*). The nutrient solutions employed were based on that used in the water cultures described in an earlier paragraph, and were .25 C, .50 C, and 2.0 C. They had total concentrations, therefore, of 364, 727, and 2908 parts per million. It is not feasible to maintain rigidly a given concentration of nutrient solution in sand cultures, but by frequent renewals it was possible to maintain the approximate concentrations. The cultures which had the .25 C solution were renewed at intervals of 7 to 10 days, the others were renewed at intervals of 14 to 21 days. The trees were planted in the sand cultures April 5, 1921, and grew for 21 months.

The leaves of the trees receiving .25 C nutrient solution were yellow-green; those receiving .5 C solution were dark green but not nearly so deep a green as the leaves of trees receiving 2.0 C solution. The rootlets in the .25 C solution when in the fresh condition appeared to be finer than in the highest concentration.

TABLE IV.—*Data on the growth of young Valencia orange trees in sand cultures to which nutrient solutions of various concentrations were added. (Each lot consisted of 6 trees, and figures represent averages.)*

		Concentration of nutrient solution		
		0.25 C	0.50 C	2.0 C
Number of leaves per tree		931	1,162	1,351
Number of yellow leaves per tree		48	57	----------
Green weight (in grams)	Leaves	----------	653	748
	Shoots	----------	290	342
	Trunk	----------	507	484
Dry weight (in grams)	Leaves	177	227	273
	Shoots	125	160	187
	Trunk	261	313	290
	Root	243	324	349
	Rootlets	300	376	318
	Total	1,106	1,400	1,417
Volume of liquids added to cultures (in liters)	Nutrient solution	253	173	162
	Distilled water	391	471	462
Volume of drainage water recovered (in liters)		224	170	171
Water transpired by trees (in liters)		420	474	452
Ratio of transpiration to dry weight	Entire tree	380	339	319
	Leaves	2,373	2,090	1,656

Table IV gives the dry weight of the different portions of the trees, and also the green weights of the tops of the trees in the .5 C and the 2.0 C cultures. The green and dry weight of the leaves and shoots increased with increasing concentration of the culture solution. The diameter and appearance of the trunk and root of the different trees were quite uniform at the time of planting. However, there must have been some initial differences in weight of the tree portions when planted, hence differences in the green or dry weights of the trunks and roots will not be emphasized. Trees receiving .5 C solution produced the largest dry weight of rootlets. The total dry weights of trees receiving .5 C or 2.0 C solution were practically identical, but the average dry weight of trees receiving .25 C solution was less than the others. The water requirement per tree in each series showed slight reduction with increasing concentration

of culture solution, but the ratio of the total dry weight of leaves and shoots to that of rootlets increased with increasing concentrations of culture solution. The increased nitrate content of the more concentrated solutions may play an important part in determining the ratio of tops to roots.

Photographs were taken of the trees just prior to their removal from the containers, nearly 21 months after the trees were planted. Plate 1, A, B, and C, shows the condition of the trees receiving different solutions, and D shows their root systems.

The per cent of ash (Table V) in the various portions of the trees is greatest in those trees receiving the double strength solution. The percentages of ash in the rootlets show the largest differences, the per cent of ash in the rootlets of the trees receiving double strength solution being about twice that of the rootlets receiving quarter or half strength solutions. The composition of the ash of the three series of trees was not materially different except in the case of PO_4. The percentages of each cation in the leaves, shoots, trunk, and root are approximately constant for the different concentrations of culture solution. The percentage of sodium in the ash of the rootlets increases with increasing concentration of the culture solution, while that of potassium is approximately constant at the two lower concentrations of culture solution, but is higher in the ash of the rootlets at the highest concentration.

Table V shows that in general there is very little change in percentage composition with increasing concentration of culture solution. The trees apparently utilized the increase of nutrient ions in large measure for increases in size instead of producing a given amount of growth and accumulating large concentrations of certain ions, as frequently happens when trees are grown in unfavorable culture solutions.

THE EFFECT OF CONCENTRATION UPON THE SOLUBILITY OF INORGANIC CONSTITUENTS OF THE DRY MATTER OF THE TREES

Table V contains data showing the soluble fraction of the constituents determined in the dry matter of all portions of these trees. The fraction dissolved in water was greatest in the rootlets, and least in the trunk. There was a tendency toward increased solubility in the ash as the concentration of the nutrient solutions was increased. Taking a general view of the data presented in Table V, it appears that an increased concentration of salts in the nutrient solution results not only in an increased concentration of ions in the ash of the trees which received them, but in an increase of the fraction which is soluble in water. The case of calcium forms a conspicuous exception to the statement just made. This accumulation of surplus materials in a soluble state should set aside certain assumptions on the principles of absorption by plants which postulate insolubility instead of solubility (*6*). Moreover, the discovery of this condition in orange trees explains certain effects which have been observed in soils of high salt content. We should especially like to emphasize the importance of the calcium relation. Previous papers by the writers (*4* and *5*) have contained data on the harmful effects of sodium salts and on the ameliorating effects of calcium salts upon orange trees. Now, if the solubility relations are maintained as concentrations increase, it is apparent that as the concentration of the harmful sodium salts increases in the trees their relative solubility increases, while as the concentration of the beneficial calcium ion increases its relative solubility decreases, except in the leaves. If this be the case, we are then in a better position to understand the results often observed when trees grow in soils of high salt content.

TABLE V.—*Composition of the ash and water solubility of inorganic constituents of dry matter of young orange trees grown in sand cultures*

	Leaves						Shoots					
	0.25C		0.50C		2.0C		0.25C		0.50C		2.0C	
	Per cent of ash	Per cent H_2O soluble	Per cent of ash	Per cent H_2O soluble	Per cent of ash	Per cent H_2O soluble	Per cent of ash	Per cent H_2O soluble	Per cent of ash	Per cent H_2O soluble	Per cent of ash	Per cent H_2O soluble
Ash as percentage of dry matter	13.73	.....	13.49	.....	14.34	.....	4.48	.....	4.30	.....	6.30	.....
Inorganic constituents of dry matter	.....	47.47	.....	54.47	.....	58.81	.....	39.48	.....	39.62	.....	38.94
Na	4.57	85.68	4.06	88.10	4.58	95.74	3.97	69.87	3.86	84.16	3.29	81.02
K	18.05	86.21	18.20	95.43	18.23	97.93	15.10	89.49	15.59	91.08	14.89	91.79
Ca	20.37	23.21	19.81	27.33	20.88	34.65	21.99	11.50	21.85	9.17	22.95	9.02
Mg	2.15	60.74	1.97	75.24	1.85	79.62	3.15	55.63	3.17	54.35	2.40	56.95
Cl	0.26	100.00	0.26	100.00	0.30	100.00	0.37	100.00	0.40	100.00	0.30	100.00
SO_4*	3.94	65.53	4.34	71.58	4.39	68.89	3.43	61.92	3.53	65.04	4.86	70.16
PO_4*	2.84	56.43	3.41	62.09	4.70	69.58	4.57	57.82	5.37	63.91	7.77	84.24

	Trunk						Root					
	0.25C		0.50C		2.0C		0.25C		0.50C		2.0C	
	Per cent of ash	Per cent H_2O soluble	Per cent of ash	Per cent H_2O soluble	Per cent of ash	Per cent H_2O soluble	Per cent of ash	Per cent H_2O soluble	Per cent of ash	Per cent H_2O soluble	Per cent of ash	Per cent H_2O soluble
Ash as percentage of dry matter	2.89	.....	2.38	.....	3.21	.....	2.42	.....	2.56	.....	2.91	.....
Inorganic constituents of dry matter	.....	30.12	.....	34.93	.....	35.02	.....	37.16	.....	35.09	.....	42.96
Na	3.53	68.62	4.44	72.35	3.42	83.99	2.92	96.39	3.29	100.00	3.71	100.00
K	12.86	87.36	15.20	87.59	13.97	93.10	14.48	92.22	13.68	92.13	15.89	94.51
Ca	19.89	7.24	20.68	6.79	22.70	6.51	18.48	6.11	16.48	5.46	17.73	6.56
Mg	1.61	44.92	1.91	40.50	1.68	57.28	1.95	32.64	1.98	83.19	2.35	45.74
Cl	0.46	100.00	0.47	100.00	0.39	100.00	0.50	100.00	0.43	100.00	0.38	100.00
SO_4*	2.82	27.78	2.89	39.94	3.75	43.36	2.02	35.81	2.62	35.47	2.68	41.16
PO_4*	3.94	66.19	5.89	72.30	6.82	80.55	6.85	57.53	7.92	55.04	8.15	70.90

	(Silica-free) Rootlets					
	0.25C		0.50C		2.0C	
	Per cent of ash	Per cent H_2O soluble	Per cent of ash	Per cent H_2O soluble	Per cent of ash	Per cent H_2O soluble
Ash as percentage of dry matter	5.03	.....	4.91	.....	9.14	.....
Inorganic constituents of dry matter	.....	64.99	.....	65.31	.....	60.77
Na	4.06	100.00	5.54	98.97	7.09	98.78
K	19.66	93.51	18.19	94.69	28.33	95.89
Ca	7.52	35.72	7.11	32.59	7.80	21.39
Mg	8.05	71.14	7.79	68.76	3.71	65.90
Cl	1.65	100.00	1.85	100.00	1.57	100.00
SO_4*	8.08	57.86	10.14	70.32	12.52	86.40
PO_4*	7.34	42.92	8.83	39.59	12.05	36.62

* These do not include organic S or P.

THE ASH OF TREES GROWN IN SOIL

The foregoing pages have discussed the composition of trees grown in sand cultures to which nutrient solutions were added. We shall present similar data upon trees grown in soil (Table VI). The results are also given for the water solubility of the inorganic constituents of the dry matter. The dry matter was placed on a quantitative filter paper in a funnel and was washed with distilled water until approximately 600–800 cc. of filtrate were obtained. After each application of distilled water to the dry matter, the distilled water was not renewed until the filtrate ceased dropping from the funnel. The filtrate was evaporated to dryness in a porcelain dish and ignited at low heat.

The ash obtained from the filtrate after leaching the dry matter of the various parts to the trees decreases as we pass from the leaves to the rootlets, where the solubility is approximately that of the shoots. The ash obtained from the filtrate of leached leaves constituted 57 per cent of the total ash found in the entire dry matter of the leaves. There is considerable water-soluble sodium in the leaves, but it remains fairly constant at a lower value in other portions of the trees. However, the actual amounts of sodium present in these trees are relatively small.

The solubility of calcium deserves particular attention, only 45.82 per cent being soluble in the leaves, 15.03 per cent in the shoots, while the trunk, root, and rootlets contained only 7 to 9.5 per cent soluble calcium.

In comparing these trees with those from the sand cultures (Table V) one sees that the percentage of potassium was lower in the trees from the soil, while that of calcium was higher. The solubility of sodium was less in the ash of the trees from the soil and that of calcium was slightly higher. With the exception of the rootlets, the sulphates and phosphates were more soluble in the trees from the soil than in those from the sand cultures.

TABLE VI.—*Data on the composition of orange trees grown in soil; analysis of the ash and the water-soluble fractions of the dry matter*

	Leaves		Shoots		Trunk		Root		Rootlets	
	Percentage	Solubility	Percentage	Solubility	Percentage	Solubility	Percentage	Solubility	Percentage	Solubility
Nitrogen in the dry matter	1.94		0.85		0.52		0.51		1.19	
Ash in the dry matter	13.96	57.02	5.90	39.10	2.34	35.71	2.37	27.42	8.06	40.66
Ash constituents:										
Na	2.06	83.30	3.71	62.82	5.42	57.24	7.59	66.74	2.91	64.87
K	8.43	99.24	9.52	97.24	12.34	96.93	8.01	92.94	13.53	95.18
Ca	28.16	45.82	28.01	15.03	23.51	8.69	19.60	7.18	18.18	9.39
Mg	2.51	85.84	2.90	67.16	2.34	48.59	1.69	36.45	2.70	67.92
Cl	0.28	100.00	0.41	100.00	0.74	100.00	1.43	100.00	4.67	99.20
SO_4	4.64	81.73	4.16	73.88	4.43	55.22	4.54	47.25	11.30	75.97
PO_4	3.29	94.58	6.50	90.09	7.71	76.96	9.32	63.64	5.18	58.55

SUMMARY

(1) The sap expressed from leaves of orange trees showed remarkable constancy in the concentration of solutes and in hydrogen-ion concentration, although grown in nutrient solutions of differing composition.

(2) Orange seedlings grown in water cultures, ranging in concentration from 364 to 3,635 parts per million of solutes, indicated that the most favorable con-

centration was near 2,200 parts per million. This was also the concentration at which the ratio of transpiration to dry weight was lowest.

(3) Young orange trees grown in sand cultures supplied with nutrient solutions containing 364, 727, and 2,908 parts per million of solutes made practically the same amount of growth in each of the last two concentrations mentioned. The percentage of ash in the dry matter was greatest in the case of trees which received the most concentrated solution. The composition of the ash showed less tendency to vary than in the case of seedlings grown in water cultures, though there were some well-defined differences in the ash of rootlets.

(4) The water-soluble inorganic constituents of the dry matter were usually greater in the case of trees which received the more concentrated nutrient solutions. Certain exceptions were found in the case of calcium. There seems to be no evidence in support of the assumption that the absorbed materials are converted into insoluble compounds as rapidly as they accumulate in the plant nor that absorption necessarily depends on the precipitation of ions within the plant. The nature of injuries which orange trees often exhibit when grown in saline soil may be related to the fact that the percentage of soluble sodium compounds appears to rise faster than the percentage of soluble calcium compounds.

(5) The solubility of the inorganic constituents of the dry matter varied considerably in various parts of the tree. The highest percentage of soluble materials was found in the dry matter of the leaves, and the next highest, with the exception of phosphates, in the rootlets. The lowest percentage of soluble materials usually occurred in the trunk and root. In other words, the greatest proportion of soluble materials was found in the parts of the tree in which the most active metabolism occurs.

LITERATURE CITED

(1) Cummins, A. B., and Kelley, W. P.
1923. The formation of sodium carbonate in soils. Calif. Agr. Exp. Sta. Tech. Paper 3, 35 p.

(2) Haas, A. R. C.
1920. Studies on the reaction of plant juices. Soil Sci. 9: 341–368, illus.

(3) Reed, H. S., and Haas, A. R. C.
1923. Growth and composition of orange trees in sand and soil cultures. Jour. Agr. Research 24: 801–815, illus.

(4) ——— ———
1923. Effect of sodium chlorid and calcium chlorid upon growth and composition of young orange trees. Calif. Agr. Exp. Sta. Tech. Paper 4, 32 p., illus.

(5) ——— ———
1923. Studies on the effects of sodium, potassium, and calcium on young orange trees. Calif. Agr. Exp. Sta. Tech. Paper 11, 32 p., illus.

(6) Truog, E.
1922. The feeding power of plants. Science 56: 294–299.

PLATE 1

A.—Orange trees in sand cultures which had received .25 C nutrient solution.
B.—Orange trees in sand cultures which had received .50 C nutrient solution.
C.—Orange trees in sand cultures which had received 2.0 C nutrient solution.
D.—The root systems of orange trees from sand cultures: No. 93 received .25 C solution; No. 99 received .50 C solution; and No. 110 received 2.0 C solution.

B
D
A
C

THE GERMINATION OF COTTONSEED[1]

By EBEN H. TOOLE, *Physiologist*, and PEARL L. DRUMMOND, *formerly Junior Seed Botanist, Seed Testing Laboratory, Bureau of Plant Industry, United States Department of Agriculture*[2]

INTRODUCTION

During 1921 and 1922, G. W. R. Davidson, of the Office of Crop Acclimatization and Adaptation Investigations of this Department, called to our attention the fact that our germination tests of cottonseed, especially of seed from Texas, gave very variable and often unaccountably low results. At about the same time the Texas Agricultural Experiment Station discussed with the Seed Testing Laboratory the difficulties of securing reliable results in making germination tests of cottonseed. We have attempted to analyze the situation and to locate the difficulties. Although all cottonseed are sensitive to conditions of germination, a good sample will germinate promptly and vigorously under a comparatively wide range of moisture and temperature conditions. Occasional samples, due to heating in storage or to other factors, contain many seeds with dead or weak embryos; such samples will not germinate vigorously under any conditions. On the other hand, a great many samples of cottonseed, when germinated by the standard method,[3] are conspicuous because of excessive mold and because of the "damping off" of the sprouts. This condition is most frequent in Texas seed of certain varieties.

VARIATIONS IN GERMINATION RESULTS

One of the great difficulties in germinating cottonseed from Texas has been the great variation in the results obtained from different tests of the same sample. Fourteen separate tests of a single sample by the standard method, all put in the germinator at the same time, gave results ranging from 34 per cent to 58 per cent; with another sample, the results varied from 14 per cent to 46 per cent. These same samples, when tested in small boxes of soil in the germinator, produced over 90 per cent of seedlings which were uniformly vigorous and fully as strong as those from samples which did not mold by the standard method. Great variations in the results of germination tests occur also when various methods are used in making the tests, as is illustrated by the average germination of 12 samples, all of one variety and received at the same time from a large seed firm. The average germination of the 12 samples was as follows: Standard method, 65.6 per cent; in soil in laboratory, 84.1 per cent; in sphagnum moss, 85.1 per cent; in soil in a cool greenhouse, 72.9 per cent; in soil in a warm greenhouse, 92.3 per cent.

---

[1] Received for publication March 17, 1924.

[2] The writers are indebted to W. L. Goss, G. W. R. Davidson, H. C. McNamara, W. W. Ballard, and B. M. King, of this Bureau, and A. H. Leidigh of the Texas Agricultural Experiment Station for aid in the field tests and for helpful suggestions during the progress of the work.

[3] As given in the "Rules for seed testing," adopted by the Association of Official Seed Analysts of North America, [4] the dry seeds are put between folds of moist cotton flannel or of moist absorbent paper toweling and kept in a germinator at the usual 20-30° C. alternation of temperatures (30° C. for 6 to 8 hours, and 20° C. for the remainder of the day).

[4] ASSOCIATION OF OFFICIAL SEED ANALYSTS OF NORTH AMERICA. RULES FOR SEED TESTING. As recommended at its 10th annual meeting, 1917. Revised 1921. (Mimeographed).

COTTONSEED OF THREE CLASSES

After much study of the problem, it was clear that in cottonseed there are three distinct classes of samples:

1. Strong samples in which a large percentage of the seeds will germinate well under a wide range of conditions (Pl. 1, A).

2. Samples which contain many dead or weak seeds which always fail to produce healthy seedlings (Pl. 2, A).

3. "Sensitive" samples, in which a large percentage of the seeds are viable and capable of producing seedlings, although there is a tendency for the seeds to succumb under unfavorable conditions of germination (Pl. 1, C).

The first two classes offer no difficulties to the analyst, so it is the third class which interests us here. It is especially important because a large per cent of the samples of some varieties received from Texas during the past two seasons have belonged to this "sensitive" class.

When sensitive samples are germinated by the standard method, the entire test is often covered by a growth of fungus, many of the young sprouts are water-soaked and decayed, and in many of the remaining seeds the hypocotyl decays before it has a chance to break through the seed coat. From the fact that other samples of the same variety, grown on the same tray, show no tendency to mold, and consistently give high germination results, it is clear that the mold and decay do not result from contamination of the substratum or of the germinator.

RELATION OF MOLDS AND BACTERIA

An examination by Dr. Chas. Dreschler, of this Bureau, of seedlings from both soil and laboratory germination tests of sensitive samples failed to reveal the presence of any organisms definitely connected with recognized diseases of cotton. However, a beginning has been made of a study of the various organisms actually present on the seeds and their possible relation to the decay of the seeds and seedlings.[5] On the surface of the seeds were found many species of bacteria, and several fungi, the most common being *Rhizopus nigricans*. Surface sterilization of the dry seeds with $HgCl_2$ (1:1,000) for 30 minutes gave completely sterile seeds. However, seeds which had been in the germinator for 24 hours or longer and were then surface sterilized in the same manner, revealed the presence within the seeds of *Rhizopus* sp. and numerous bacteria—all common soil organisms.

SENSITIVE SAMPLES NOT THE RESULT OF "HEATING" IN STORAGE

It was at first suspected that the tendency of some samples of cottonseed to mold and decay during germination was due to "heating" in storage. Although a study of the moisture content and temperature of stored cottonseed in Texas in the fall of 1922 gave no evidence of the occurrence of "heating" of the seed, sensitive samples were very prevalent in that season. Damage to cottonseed from "heating" does occur, as has been described in a report of the Arkansas Agricultural Experiment Station,[6] but "heating" of the stored seed is not the cause of the "sensitive" condition of much Texas cottonseed.

---

[5] The writers are indebted to N. R. Smith, of this Bureau, for help with materials and methods, and for the identification of organisms.

[6] KNAPP, B.—THE EFFECTS OF METHODS OF HARVESTING AND STORING ON COTTONSEED. Ark. Agr. Exp. Sta. Bul. 181: 23. 1922.

TEMPERATURE FOR GERMINATION OF COTTONSEED

As a result of a study of the effect of temperature on the laboratory germination of cottonseed, it was concluded that the excessive molding of sensitive samples is not dependent on the temperature of germination, and that the usual 20°–30° C. (68°–86° F.) alternation (30° C. for 6 to 8 hours and 20° C. for the remainder of the day) is probably the best temperature of cottonseed germination. Cottonseed can be germinated satisfactorily at 20° C. (although slowly) at 25° C. (77° F.) and at a 15°–35° C. (59°–95° F.) alternation (35° C. for 6 hours and 15° C. for 18 hours).

A comparison of the usual 20°–30° C. alternation with a similar 18°–32° C. alternation and with the reverse alternation (18° C. for 6 to 8 hours and 32° C. for the remainder of the day) indicated that the usual 20°–30° C. alternation is the most satisfactory both for the rate and for the completeness of germination.

DEMONSTRATION OF "LIVE SEEDS" IN SENSITIVE SAMPLE

As has been indicated above, the "sensitive" samples have been designated as such because a large percentage of the seeds can by special methods be demonstrated to be viable although germination by the standard method gives a low and variable result. It was found that the "live seed" in these sensitive samples could be determined by any one of the following special methods: By testing the seeds in soil with controlled moisture content and controlled temperatures; often by soil tests in the greenhouse; by tests in sphagnum moss; to some extent by "presoaking" the seeds; by surface sterilizing the seeds; and by simply wetting the fuzz of the seeds before putting them to germinate. It would seem that any method which will get water into the seeds quickly and start germination promptly will prevent the development of mold and the decay of the sprouts.

With the soil method, it is difficult to obtain a soil which will uniformly supply the seeds with sufficient moisture. Greenhouse testing in soil is satisfactory if the temperature can be maintained between 60° and 80° F. Surface sterilization of the seeds, either by a dilute solution of mercuric chlorid or by dusting with dry copper carbonate powder (as suggested by Prof. A. H. Leidigh of the Texas Agricultural Experiment Station) will usually control the mold and decay during the germination of these sensitive samples. However, the writers have always hesitated to use a sterilizing agent in making tests of the germinating power of commercial seeds. Although for the prompt germination of cotton the seeds must absorb a large amount of water quickly, an excess of water interferes with germination, probably by limiting the supply of oxygen. For this reason, presoaking the seeds is uncertain, as often a part of the sample is rendered dormant by the process. The simplest and most successful treatment of sensitive samples was found to be a thorough wetting of the fuzz of the seeds before they were put to germinate. This has been designated the "prewetting method." This insures prompt germination of the seeds and in most cases entirely prevents the spread of mold and the decay of the young sprouts.

A few exceptional samples have been encountered which behave in a peculiar manner when tested by the prewetting method. In these samples, many of the seeds remain in a dormant condition in the germinator, as they do when soaked too long in water. In these cases, the total germination is greater by the standard method. Wherever it has been possible to trace the history of such samples, it has been found that the development of the seed has been checked either by weather conditions or by insect attack. Fresh seeds, which still contain a large amount of moisture, behave in the same way.

The difference in the germination results obtained by the two methods is indicated best by a few examples. A typical sensitive sample germinated 71 per cent and 54 per cent in two tests by the standard method; however, the sample contained a large per cent of live seed as indicated by germinations of 95 and 94 per cent, respectively, by the prewetting method. On the other hand, a typical strong sample germinated 95 per cent by the standard method and 98 per cent after prewetting. Prewetting the seed not only prevents the molding of sensitive samples, but greatly hastens the germination of all samples (Pl. 1, A and B). Evidence of the prevalence of sensitive samples, and of the effect of prewetting is shown by the results of one hundred and fourteen tests taken at random from the reports of the Texas Branch of the Seed Testing Laboratory; by the standard method the one hundred and fourteen tests averaged 66.9 per cent germination, by the prewetting method 87.5 per cent. Of these 114 tests, 93 were of typically sensitive samples. Of the remaining 21 tests, 4 were of samples from Louisiana, 7 were separate tests of a single strong sample, 6 were of other strong samples, and 4 indicated samples with a large proportion of dead seeds.

The effect of prewetting on the germination of the three types of samples is shown in Plates 1 and 2, Plate 1, A and B, show No. 483—a strong sample. The germination by the standard method (A) is as good as by the prewetting method (B), although slower. Plate 1, C and D, shows No. 446—a sensitive sample. The excessive mold when germinated by the standard method (C) is typical of this type of sample. The vigorous clean germination after prewetting (D) indicates the high percentage of live seeds. In contrast to the above, the poor germination by both methods (A, standard method) (B, prewetting method) of a typical weak sample, No. 375851, is shown in Plate 2, A and B.

HOW TO USE THE PREWETTING METHOD

In the use of the prewetting method, the seeds are put in a glass jar with enough water to cover. This is shaken vigorously until the seeds appear uniformly dark colored, indicating that all the air has been displaced from the fuzz and that the surfaces of the seeds are thoroughly wet. Then they are drained and the film of excess water blotted off (on account of the danger of causing dormancy because of too much water). The seeds are then put between folds of wet paper towels and germinated at the usual 20°–30° C. (68°–86° F.) alternation. It has not been possible to standardize the amount of shaking, as some samples are more difficult to wet than others.

During the course of these investigations, the substratum for germinating cottonseed was changed from cloths to an absorbent quality of paper toweling. The germination has been found practically identical with either material, but the convenience and economy of the paper makes it very desirable.

FIELD BEHAVIOR OF SENSITIVE SAMPLES

Although we found that the sensitive samples contained a large proportion of live seed, which could be germinated by various simple methods, we were uncertain how to interpret to the planter of the seed the results of the germination tests. It was necessary to know the behavior of sensitive samples in comparison with strong samples under different field conditions. Accordingly, we selected, for field study, six samples which had been tested in the laboratory under a wide range of conditions. Arrangements were made through the cooperation of the Office of Crop Acclimatization and Adaptation Investigations of this Bureau and of the Texas Agricultural Experiment Station, to have these samples planted at Lawton, Okla.; Greenville, San Antonio and College Station,

Tex., as well as at the Arlington Experiment Farm near Washington, D. C. Five hundred seeds of each sample were spaced about two inches apart by hand at each planting. From three to six plantings, about a week apart, were made at each station. It was hoped in this way to study the effect of a variety of environmental conditions on the field germination of sensitive samples. The laboratory behavior of the six samples used in the field tests is described in Table I.

TABLE I.—*Description of the six samples of cottonseed used in the field tests*[a]

No.	Variety	Source	General behavior in laboratory tests	Germination just before sending out for the field tests		
				Standard method	"Prewetting" method	In soil in germinators
				Per cent	*Per cent*	*Per cent*
483	Lone Star	Clarksville, Tex.	Uniformly high germination; no tendency to mold.	94	97	95
499	do	Manchester, N. C.	Generally high germination; occasionally slight tendency to mold.	93	96	94
G-40	do	Greenville, Tex.	Somewhat "sensitive"	84	91	93
446	do	Mesquite, Tex.	Very "sensitive." Heavy mold when tested by standard method.	64	93	97
C-7	Mebane	Lockhart, Tex.	"Sensitive." Considerable mold when tested by standard method.	71	95	97
5114	do	do		79	97	99

[a] All seed of 1922 crop except No. 446, which was grown in 1921 and stored in the laboratory for about eighteen months.

When tested in soil in the laboratory or by the prewetting method, these samples were equally good, but when tested by the standard method, two samples were strong and the other four were sensitive in various degrees.

Their relative behavior under field conditions is summed up in Table II.

TABLE II.—*Average field germination of the six selected samples of cottonseed. The germination for each sample is averaged from several successive plantings at each of five stations*

Station	Number of plantings averaged	Average germination for each sample					
		Sample No. 483	Sample No. 499	Sample No. G-40	Sample No. 5114	Sample No. C-7	Sample No. 446
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Arlington, Va.	5	72.6	62.2	61.8	55.9	50.9	40.2
		[a] (64–80.8)	(45.8–78)	(52.8–72.6)	(51–60.6)	(41–64.8)	(32.6–53)
Lawton, Okla.	5	70.9	58.8	58.4	57.5	61.9	31.4
		(58.8–75.8)	(51.2–64.4)	(47.4–66.8)	(44.2–68.8)	(56.6–73.2)	(27–40.8)
Greenville, Tex.	6	70.6	63.7	61.1	52.7	48.2	37.1
		(61.4–85)	(37.8–79.2)	(48–66.2)	(44.2–59.4)	(31–58.4)	(30–43)
San Antonio, Tex.	3	83.3	74.9	74.2	66.3	68.9	45.5
		(78.4–86.6)	(72–79.4)	(65.6–80.2)	(60.2–69.8)	(60.2–73.8)	(34.4–58.4)
College Station, Tex.	3	70.3	77.1	64.5	61.6	67.2	36
		(68.8–71.4)	(69.6–87.2)	(59.2–71.8)	(58–65.6)	(56–79.2)	(25–49.6)
Average of all plantings.	22	72.8	65.6	62.9	57.6	57.4	37.6
		[a](58.8–86.6)	(37.8–87.2)	(47.4–80.2)	(44.2–69.8)	(31–79.2)	(25–58.4)

[a] The numbers in brackets represent the extremes of germination of the individual plantings that enter into the average.

The results of the individual plantings at each station showed considerable variation, depending on the conditions at the time of planting. This is especially noticeable in the germination of the sensitive samples. The germination results for each of the successive plantings at Greenville, Tex., are given in Table III as illustrations of the variations obtained.

TABLE III.—*Summary of the average field germination of cottonseed for successive plantings at Greenville, Tex., 1923*

Sample No.	Apr. 9	Apr. 16	Apr. 23	May 2	May 7	May 15	Average
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
483	61.4	85.0	73.6	79.8	62.4	61.4	70.6
499	37.6	75.6	51.8	79.2	78.4	59.6	63.7
G–40	57.0	64.4	66.2	65.4	65.6	48.0	61.1
5114	44.2	52.8	54.4	55.6	59.4	49.8	52.7
C–7	31.0	44.2	52.6	56.0	58.4	47.2	48.2
446	31.2	38.6	38.8	43.0	41.4	30.0	37.1

In the results of this field study of cottonseed germination, a few points stand out clearly. The strong samples, especially No. 483, gave consistently better stands than the sensitive samples. Sample No. 446 was consistently the lowest germinator in the field, as it had been in the laboratory. It is clear that, on the whole, these sensitive samples are not so poor as would be indicated by the result of the standard test. They are capable of producing a good stand of plants when field conditions are favorable. On the other hand, they are not so valuable as the strong samples, as would be indicated by the result of the test by the pre-wetting method, for they were decidedly less able to withstand extremes of temperature or of moisture. In this connection, it should be emphasized that even the best samples of cottonseed are very sensitive to conditions of germination in the field and may fail to give a stand under unfavorable weather or cultural conditions.

REPORTS ON THE LABORATORY GERMINATION

As has been pointed out, a test by the prewetting method will determine the number of live seed and distinguish sensitive samples from samples of truly low viability. Although the standard method gives very variable results with these samples, it distinguishes them from the strong samples, and, in connection with the determination of the live seed, gives a very rough idea of the quality of the sample. With these points in mind, the following provisional procedure for reporting cottonseed germination has been adopted by our laboratory.

Each sample is tested by both the standard method and the prewetting method. In addition to the report of the standard test, the result of the prewetting test is reported as "live seed." Each report is accompanied by a brief explanation of the reason for the two tests and their significance. This same procedure is being followed by the Texas State Department of Agriculture.

MOISTURE CONTENT OF STORED COTTON SEED

The moisture content of the stored seed has been found to be an important factor in the germination of sensitive samples. As the moisture content falls below about ten per cent there is a gradual increase in the tendency of the sample to mold and decay during germination. Moreover, if the seeds dry out until the moisture content is between five and six per cent, there is a marked development of "hard seeds," which fail to take up water at all. A sample from Sacaton, Ariz., was so dry when received that it contained

many hard seeds. Also samples which are kept in a heated laboratory rapidly lose moisture and give increasing trouble in germination. The moisture content of this dry cottonseed can be raised by sprinkling the seed with a small amount of water (about two gallons of water to one hundred pounds of seed), which should be evenly distributed by a thorough mixing of the mass of seed. Then the pile of seeds should be covered with canvas and left for a week to ten days. This moistened seed should be used promptly and not kept for any length of time. Under this treatment the hard seeds are rendered capable of taking up water from the soil, and the rapidity and uniformity of germination are greatly increased. (See Pl. 2, C.) An unusually dry sample, when treated as described and planted in a rather dry soil in the field, germinated 65 per cent in 7 days, while the untreated portion germinated only 29 per cent in the same time.

Prewetting or presoaking cottonseed before planting in the field has not increased the field germination where the soil moisture was good. However, it greatly hastens germination where the soil moisture is not sufficient for the prompt sprouting of untreated seed but where there is enough moisture to maintain the seedlings after germination. From the experience of H. C. McNamara in Texas and in New Mexico, it is suggested as a promising method to be used with caution under special conditions.

SOME SUGGESTIONS FROM THE PRELIMINARY STUDY

In a discussion of the results of this preliminary study two viewpoints are to be kept in mind—that of the seed analyst and that of the grower or planter.

Any seed analyst who may have occasion to make germination tests of cottonseed, at least of that grown in the Southwest, should recognize the existence of sensitive samples. It would seem to be only fair to the seed grower and the planter of the seed to distinguish these sensitive samples from undoubtedly strong samples and from samples containing a considerable percentage of dead seeds. This distinction may be made by making two germination tests as outlined above. However, the report of the test should indicate that the sensitive samples are not so valuable as the strong samples. Analysts should endeavor to test samples promptly when received, or, if this is not possible, the samples should be protected from the loss of moisture, since excessive drying increases the tendency of sensitive samples to mold during germination, and the results of the test will not represent the true condition of the original lot of seed.

Both the grower and the planter of cottonseed should recognize that all cottonseed, even of the highest quality, is more sensitive to conditions of germination, both in the laboratory and in the field, than the seed of most field crops. It is important that great care should be exercised both in harvesting and in storing to insure sound seed. However, no information is now available to indicate how the occurrence of sensitive seed may be avoided. This phase of the subject is being worked on at present. When the seed analyst's report indicates that planting seed is of the sensitive class, it should be planted only when the temperature and moisture of the soil are very favorable for the germination of cottonseed.

Cottonseed that is very dry will not germinate as promptly or vigorously as that containing a higher moisture content (approximately 12 per cent) at the time it is planted. On the other hand, it is undoubtedly true that during storage the dryer seed will keep its vitality longer. If, at planting time, the seed is excessively dry, it is suggested that the promptness of germination can be increased by artificially raising the moisture content of the seed as described above. In most sections the increased moisture of the atmosphere which prevails in the spring will be sufficient to bring the seeds to a suitable condition for planting.

PLATE 1

Comparative germination of strong (A and B) and sensitive (C and D) samples by the standard and "prewetting methods."

A.—No. 483, standard method.

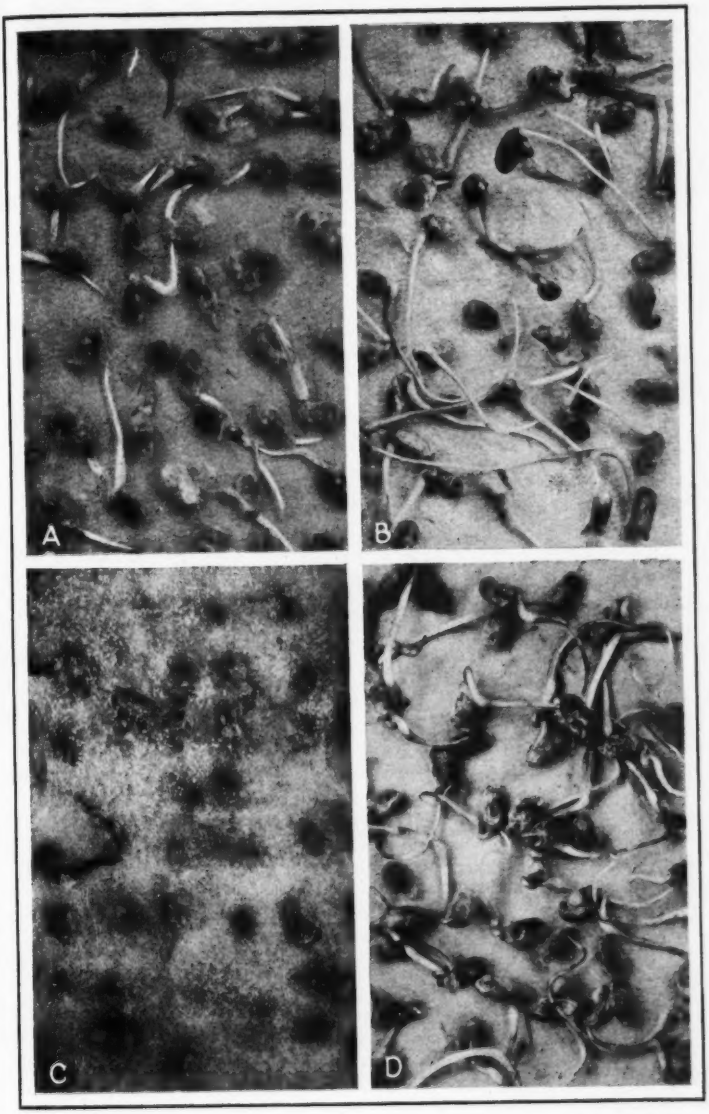
B.—No. 483, prewetting method.

Note that germination is good by either method, but is somewhat more advanced by the prewetting method.

C.—No. 446, standard method.

D.—No. 446, prewetting method.

Note the high percentage of "live seed" indicated by the prewetting method and the low germination and excessive growth of mold when this "sensitive" sample is germinated by the standard method.


A
B
C
D

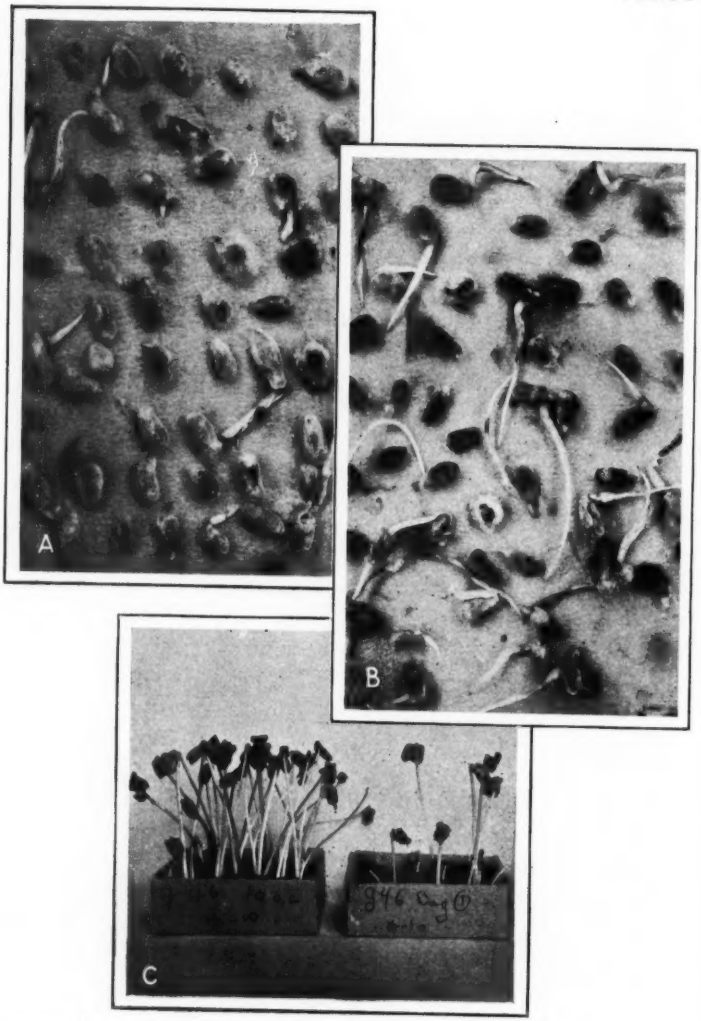

A
B
C

PLATE 2

Germination of a weak sample (A and B) and relation of moisture content of seed to germination (C).

A.—No. 375851, standard method.

B.—No. 375851, "prewetting" method.

The seeds which do not germinate by the standard method are dead, and germination is not improved by the prewetting method.

C.—No. G–46, germinated in soil in the germinators.

The seed planted in the box on the right was very dry when received from Sacaton, Ariz. (Moisture content, 5.7 per cent.) The seed planted in the box on the left was from the same lot, but the moisture content had been raised to 14.7 per cent by the method described in the text (p. 291).

THE SALT CONTENT OF COTTON FIBER[1]

By THOMAS H. KEARNEY, *Physiologist in Charge, Alkali and Drought Resistant Plant Investigations,* and C. S. SCOFIELD, *Agriculturist in Charge, Western Irrigation Agriculture, Bureau of Plant Industry, United States Department of Agriculture*

It has been reported that the fiber of Pima cotton, a long staple variety of the Egyptian type grown under irrigation in Arizona, is difficult to spin when the humidity of the air is high. The suggestion has been made that this might be due to a high salt content of the fiber, attributable to the fact that irrigated land in the Southwest often has a higher salt content than most of the soils on which cotton is grown in the Southern States.

Determination of the actual salt content of Pima fiber therefore seemed advisable. For this purpose samples of this cotton were collected in 1923 at the United States Field Station at Sacaton, Ariz., together with samples of other types of cotton grown at the same station. Samples (for which the writers are indebted to D. M. Simpson of the Office of Crop Acclimatization and Adaptation, Bureau of Plant Industry, United States Department of Agriculture), were also obtained from James Island, S. C., to permit a comparison of eastern-grown with Arizona-grown cotton.[2] In order to avoid contamination with dust, the fiber was extracted from bolls which were mature, as shown by the fact that they were beginning to crack at the tip, but in which there had been no opportunity for dust to accumulate.

The fiber which enters commerce is picked from fully open bolls and usually accumulates some dust before it is gathered. If commercial Pima cotton showed an abnormally high salt content, this might be due to the deposition of dust on the fiber in the open bolls, rather than to absorption of salt from the soil solution through the roots of the plant. To test this possibility a sample of dusty Pima fiber was collected from fully open bolls at the edge of a field near a road.

Determinations of salt content of the fiber were made on samples collected from the following eight plantings of cotton, the fiber having been extracted from ripe but unopen bolls in all cases except A:

A.—Pima Egyptian, open bolls, fiber dusty, Peoria, Ariz.
B.—Pima Egyptian, plants stunted by alkali, Sacaton, Ariz.
C.—Pima Egyptian, well-grown plants in good soil, Sacaton, Ariz.
D.—Sea Island cotton, growing adjacent to C.
E.—Meade Upland cotton, growing adjacent to D.
F.—Lone Star Upland cotton growing near C, D, and E.
G.—Sea Island cotton from James Island, S. C.
H.—Meade Upland cotton from James Island, S. C.

Each sample of fiber was divided into two subsamples, one being used for determination of the water soluble salt content and the quantity of ash not soluble in water, the other for determination of the total ash.

[1] Received for publication Mar. 20, 1924.

[2] The soil of the low-lying islands off the coast of South Carolina probably has a somewhat higher content of sodium chlorid than most cotton soils of the Southern States, but the locality where these samples were collected is typical of the area which produces the choice "crop lot" Sea Island cotton.

The water extracts were prepared by soaking 10 gm. of the dry fiber in 100 cc. of distilled water. After 24 hours' immersion, 5 cc. of the extract was withdrawn and was made up to a volume of 50 cc. by the addition of distilled water. The electrical resistance of the diluted extract was then determined and from the resistance, corrected to a temperature of 60° F., the salt content was computed as a percentage of the original 10 gm. of dry fiber.[3]

The fiber used in preparing the extracts was then washed thoroughly with distilled water to remove all water soluble salts, dried and ashed and the weight of the ash was computed as a percentage of the dry weight of the fiber.

From each of the subsamples which had not been leached, 10 gm. of dry fiber were ashed and the ash content of each subsample was computed as a percentage of the dry fiber.[4]

The results of the several determinations are given in Table I.

TABLE I.—*Water-soluble salts and ash in samples of cotton fiber, all values being given as percentages of the dry weight of the fiber*

Samples of fiber	Water-soluble salts, computed from electrical resistance	Ash residues of water-washed samples	Sums of values in columns 1 and 2	Ash residues of unwashed samples	Volatile salts (values in column 3 minus values in column 4)
ARIZONA SAMPLES					
A.—Pima from open bolls	1.57	0.38	1.95	0.99	0.96
B.—Pima from unopen bolls, alkali soil	1.73	.28	2.01	1.44	.57
C.—Pima from unopen bolls, good soil	1.58	.22	1.80	1.23	.57
D.—Sea Island from unopen bolls, good soil	1.30	.31	1.61	1.05	.56
E.—Meade from unopen bolls, good soil	1.39	.36	1.75	1.33	.42
F.—Lone Star from unopen bolls, good soil	1.53	.22	1.75	1.03	.72
SOUTH CAROLINA SAMPLES					
G.—Sea Island from unopen bolls	1.44	.20	1.64	1.10	.54
H.—Meade from unopen bolls	1.29	.21	1.50		
Average of all samples	1.48	.27	1.75	1.17	.62

It is evident from the data given in Table I that, as grown on similar soil in Arizona, the salt content of the fiber of Pima cotton (Sample C) is not appreciably higher than that of Sea Island and Upland cottons (Samples D, E, F).[5] Nor do Sea Island and Upland cottons (Samples D and E) grown on irrigated land in Arizona contain appreciably more salt than cotton of the same varieties (Samples G and H) grown in South Carolina. There is somewhat more salt in fiber of Pima cotton from a very salty field (Sample B) than in fiber of the same variety from good soil (Sample C). It is further shown that Pima fiber which has been exposed to dust (Sample A) did not have a higher salt content than fiber of the same variety from unopen bolls (Sample C).

The data in Table I show that on the average 85 per cent of the total salts and ash in cotton fiber is water-soluble material, while 67 per cent is nonvolatile material.

[3] The table used in computing the salt content from the electrical resistance, corrected to 60° F., is given by DAVIS, R. O. E., and BRYAN, H., THE ELECTRICAL BRIDGE FOR THE DETERMINATION OF SOLUBLE SALTS IN SOILS. U. S. Dept. Agr. Bur. Soils Bul. 61, p. 27-29. 1910.

[4] The writers are indebted for the ash determinations to J. W. McLane of the Office of Biophysical Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

[5] On the other hand, Harris and his associates have found the leaf tissue fluids to contain significantly higher quantities of total electrolytes and of chlorids in Pima Egyptian than in Upland cottons.

HARRIS, J. A., and others. THE TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTONS AND THEIR F_1 HYBRID. Jour. Agr. Research 27: 267-328, illus.

——— LAWRENCE J. V., and LAWRENCE, Z. W. THE CHLORID CONTENT OF THE LEAF TISSUE FLUIDS OF EGYPTIAN AND UPLAND COTTONS. Jour. Agr. Research. [Not yet published.]

The further question arises whether the slightly higher salt content of Pima fiber grown in saline soil (Sample B) as compared with that of fiber from nonsaline soil (Sample C) might result in an appreciably greater hygroscopicity, which might affect the spinning properties of the fiber. Also, it may be asked whether the hygroscopicity is reduced by removal of the water-soluble salts.

To answer these questions another portion of each sample (B and C) was divided into three subsamples of approximately equal weight. These were dried at a temperature of 228° F. until no further loss of moisture occurred and the dry weight was determined. The samples were then exposed to an atmosphere having a relative humidity of 65 to 70, at a temperature of 70° F., until they showed no further increase of weight. Their moisture content was then computed by subtraction of the dry weight from the final moist weight. They were next soaked 24 hours in distilled water, washed thoroughly with distilled water to remove the soluble material and dried to constant weight. The washed samples were then exposed to a moist atmosphere under precisely the same conditions as before, and when they ceased to gain weight, their moisture content was again computed. The quantities of water absorber hygroscopically, before and after washing, stated as percentages of the dry weights of the samples, are given in Table II.[6]

TABLE II.—*Dry and moist weights and percentages of water absorbed hygroscopically, before and after removal of the water-soluble salts, of samples of Pima cotton grown on saline and nonsaline land at Sacaton, Ariz.*

Sample and subsample	Before washing			After washing		
	Dry weight	Weight after exposure to a moist atmosphere	Percentage of water absorbed	Dry weight	Weight after exposure to a moist atmosphere	Percentage of water absorbed
B.—From saline soil:	*Grams*	*Grams*		*Grams*	*Grams*	
1	2.86	3.10	8.4	2.76	2.98	8.0
2	2.86	3.10	8.4	2.76	2.98	8.0
3	2.86	3.10	8.4	2.77	2.99	7.9
Average	2.86	3.10	8.4	2.76	2.98	8.0
C.—From nonsaline soil:						
1	2.87	3.10	8.0	2.80	3.01	7.5
2	2.86	3.10	8.4	2.76	2.99	8.3
3	2.85	3.10	8.8	2.76	2.99	8.3
Average	2.86	3.10	8.4	2.77	3.00	8.0

Comparison of the results, as given in Table II, shows that the capacity of Pima cotton fiber for hygroscopic absorption of water is not materially affected by removal of the readily water-soluble salts, the unwashed samples having absorbed, on the average, only 0.4 per cent atmospheric moisture more than the washed samples. It would seem, therefore, that the hygroscopicity of the salts present in the unwashed fiber is practically negligible. It is equally clear that the fiber produced by plants growing in saline soil (Sample B) had no higher capacity for hygroscopic absorption of moisture than the fiber produced by plants growing in nonsaline soil (Sample C).[7]

It is concluded that the difficulties said to be encountered in spinning Pima cotton under certain conditions of the atmosphere are not attributable to the salt content of the fiber.

[6] The writers are indebted to Horace H. Willis, of the Office of Crop Acclimatization and Adaptation Investigations, Bureau of Plant Industry, U. S. Department of Agriculture, for the determinations of dry and moist weight.

[7] The loss of weight of the samples as a result of washing averages 0.10 gm. or 3.5 per cent of the dry weight before washing (Table II). The content of electrolytes in Samples B and C, as given in Table I, column 1, averages 1.65 per cent of their dry weight. It appears, therefore, that electrolytes constituted approximately one-half of the water soluble material in these samples of cotton fiber.

ADDITIONAL COPIES

OF THIS PUBLICATION MAY BE PROCURED FROM
THE SUPERINTENDENT OF DOCUMENTS
GOVERNMENT PRINTING OFFICE
WASHINGTON, D. C.
AT

10 CENTS PER COPY

SUBSCRIPTION PRICE, $4.00 PER YEAR (DOMESTIC)
$5.25 PER YEAR (FOREIGN)

▽